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THE NERVE CELLS IN THE SPINAL GANGLIA

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INTRODUCTION

Recently Kiss (1932, 1933 *a*, 1933 *b*), on the basis of his prolonged osmic acid technique, has described three types of nerve cells in the spinal ganglia: (1) large round pale cells, (2) small round pale cells, and (3) dark "multipolar" cells. In an extensive inves'igation of both spinal and sympathetic ganglia in a large series of vertebrates, Kiss found that in the sympathetic ganglia only cell types (2) and (3) are present, and that cells of type (3) (dark "multipolar") are numerous in sympathetic ganglia but few in number in the spinal ganglia. He suggests that the dark-stained "multipolar" nerve cells are efferent sympathetic in nature, the large round pale cells somato-sensory and the small round pale cells viscero-sensory. This functional interpretation of the nerve cells in the spinal ganglia is opposed to the general conception of the nature of these ganglia, and the validity of the morphological findings by the use of a prolonged osmic acid technique has been seriously questioned (Fisher and Ranson, 1933; Levi, 1932).

The present authors have separately confirmed some of the morphological findings of Kiss by the use of different techniques (Bacsich, 1932; Blair and Davies, 1933, 1935), and the present paper records the results obtained from a study in collaboration of the spinal ganglia of the cat by means of osmic acid, toluidin blue and various silver methods. No conclusions are reached concerning the functional significance of the various types of nerve cells to be described, and the following remarks are mainly devoted to observations on the histological characters exhibited by the nerve cells of the spinal ganglia with the various techniques employed. Experimental studies designed to elucidate the functional significance of the cells are in progress.

THE CELLS OF THE SPINAL GANGLIA OF THE CAT

The spinal ganglia, removed carefully and without crushing from freshly killed young animals, were treated by one or other of the following methods: (*a*) some ganglia were fixed in 10 per cent. formalin (4 per cent. formaldehyde), treated by the prolonged osmic acid method of Kiss and embedded in celloidin-paraffin (Apáthy's method); (*b*) others were fixed in 10 per cent. formalin and stained by the toluidin blue method described by one of the present authors (P. B. 1932); (*c*) others were fixed in 20 per cent. neutral formalin and subjected to a pyridine-silver technique which two of the present authors (D. M. B. and

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F. D. 1935) evolved in the study of the conducting system of the heart and its nerve supply, and which was found to stain some of the intracardiac nerve cells an intensive brownish black and others a pale yellow. Serial sections of entire ganglia were cut.

With each of the above techniques, three distinct types of nerve cells can readily be recognised in virtue of their size, shape and depth of staining (Plate I, figs. 1, 2; Plate II, fig. 8; Plate III, fig. 13).

Type 1. These are large cells, round or slightly oval in shape, with a diameter commonly of 75–100 μ. They are more numerous than the cells of type 2, although the relative proportion of all types of cells varies in spinal ganglia from different spinal levels of the same animal. The cell body (type 1) usually completely fills the surrounding nucleated capsule with no distinct space between. No satellite cells, other than those of the nucleated capsule, were observed around these nerve cells. With the osmic acid technique the nerve cell is pale grey with a finely stippled texture; the reticular apparatus of Golgi is usually evident (Plate I, fig. 3). With the toluidin blue stain the cell body has a pale blue tint and is stippled with very finely granular tigroid material of a denser blue colour (Plate III, figs. 14, 15). With the silver technique the cell body has a pale yellow colour and is very finely granular in appearance (Plate II, figs. 8, 9).

Type 2. These are small cells, round in shape, whose average diameter is slightly more than one-half of that of the large pale cells. They are a little less numerous than the cells of type 1. The cell bodies completely fill their surrounding nucleated capsules, and again no subcapsular satellite cells were observed. They present much the same staining characters as described above in the case of the large round cells. The Golgi reticular apparatus is rendered evident by the osmic acid technique (Plate I, fig. 3; Plate II, figs. 8, 9; Plate III, fig. 13).

Type 3. These cells are generally intermediate in size between types 1 and 2. They are of multangular form, and a "space" intervenes between the cell bodies and their nucleated capsules. The surface of the nerve cell presents numerous indentations which, for the most part, are seen to be occupied by satellite cells (Plate I, fig. 4; Plate III, fig. 16). These appear in places to be continuous superficially with the nucleated capsule, though elsewhere they are definitely subcapsular in position, occupying the "space" between capsule and cell body. With the prolonged osmic acid technique the cell body is darkly stained and is seen to contain deeply stained coarse granules (Plate I, figs. 3, 4). The cytoplasm between the granules has a distinct brownish tint. The reticular apparatus of Golgi is usually evident in these cells and is of a coarser nature than that seen in nerve cells of types 1 and 2 (Plate I, figs. 3, 4). The toluidin blue stain reveals coarse, deep blue tigroid bodies in the cytoplasm (Plate III, figs. 14, 16). With the silver technique the cells are stained a deep brown colour in which little or no detail of structure can be made out visually. Plate II, fig. 10, is a photomicrograph, taken on a panchromatic plate, of a dark and

a pale cell, which reproduces fairly accurately what is observed under the microscope. Plate II, fig. 11, is a photomicrograph of the same section on an infra-red sensitive plate which reveals a reticular structure of the cytoplasm of the dark-stained cell (Blair and Davies, 1933).

The dark- and pale-stained cells are also readily differentiated by the Cajal technique (1922) in frozen sections (Plate III, fig. 12). It is interesting that this technique was recommended by De Castro (1922) for the study of sympathetic ganglia.

With regard to the polarity of the nerve cells in the spinal ganglia, Kiss called the dark-stained cells, revealed by his osmic acid technique, "multipolar", but in a personal communication with the present authors he agreed that the term multangular is preferable as applied to the form of the cell body of type 3, and it was really in this sense that he employed the term "multipolar". Earlier workers, like Disse (1893), Spirlas (1896), Dogiel (1896), von Lenhossék (1896), Cajal (1906), Marinesco (1906, 1907), Nageotte (1907) and Levi (1907), have described true multipolar cells in the spinal ganglia, and the present authors thought at first it might be revealed by the silver technique that the dark-stained multangular cells of the osmic acid method were truly multipolar nerve cells. This, however, was found not to be the case. Various silver methods were employed (e.g. Ranson, Bielschowsky with and without gold toning, Gross's modification of Bielschowsky, Golgi, Schultze, Cajal, etc.) which confirmed the fact elicited by the formalin-pyridine-silver technique that multipolar nerve cells in the posterior root ganglia are very few in number, much fewer than the number of dark-stained, multangular cells revealed by the osmic acid and toluidin blue methods. A few round pale cells (large and small) were found to be multipolar, as were also a few dark multangular cells. Multangularity of the cell body does not therefore necessarily signify multipolarity of the cyton. In the sympathetic ganglia, on the other hand, the cells which are stained dark by the osmic acid method were readily observed when stained by the silver methods to be multipolar (Plate III, fig. 17). The vast majority of both pale and dark nerve cells in the spinal ganglia were found to have one process only, and, although in many cases it is possible to determine in the sections that these cells are pseudo-unipolar in type (i.e. the single process is seen to divide after a short course from the cell body), it is evidently impossible to exclude the presence of true unipolar cells.

DISCUSSION

The existence of these three types of nerve cells in the posterior root ganglia has been challenged on various grounds. The suitability of the osmic acid technique has been criticised in particular by Levi (1932), but, as stated above, other techniques reveal similar morphological features in the three types of cells. The technique of Kopsch (1926) also involves the prolonged use of osmic acid, and this worker has concluded that osmic acid is not only suitable for demonstrating the Golgi reticulum but excellently preserves all other cell

constituents. Furthermore, it is generally agreed that the Golgi apparatus is very sensitive to injury, and, in this connection, Dr E. S. Horning, of the Imperial Cancer Research Fund Laboratory, who has studied the appearance of the Golgi apparatus in cells under normal and toxic conditions (1934), was good enough to examine some of our preparations; he thought that the Golgi apparatus in our specimens (pale and dark cells) was normal and indicated healthy cells. As formalin is not the best fixative for the purpose of displaying the Golgi reticulum, Dr Horning subjected some of the ganglia which we removed to Da Fano's technique (1925) (Plate I, fig. 5), which involves fixation in cobalt nitrate followed by silver impregnation, and to Nassonov's modification of the Kolatchev method (1928). From the clear demonstration of the Golgi apparatus he thus obtained, Dr Horning is of the opinion that the coarser and denser Golgi network in some of the cells indicates a separate morphological type of nerve cell; these cells in size and shape corresponded to the cells staining dark with our techniques. In the specimens prepared by the osmic acid method of Kiss, the present writers, as described above, observed that the dark multangular nerve cells contained a denser and coarser Golgi apparatus than that present in the pale-stained cells (Plate I, fig. 3). Dr Horning also subjected sections of posterior root ganglia to the micro-incineration process of Policard (1929) which he has elaborated and extended. The mineral ash of the nerve cells thus obtained, when examined by dark ground illumination, reveals differences in the constitution of the various nerve cells. The large and small round cells have a fine powdery ash (Plate II, fig. 6), whereas the multangular cells have a coarser, "lumpy" ash (Plate II, fig. 7). The ash of the satellite cells on the surface of these cells is also revealed. Dr Horning is convinced that the results of the micro-incineration method and the Da Fano technique indicate the presence in the spinal ganglia of cells of different morphological types.

Recently Fisher and Ranson (1933) criticised the findings of Kiss, and maintained that the depth of staining of the nerve cells of the spinal ganglia by the osmic acid method depends on the amount of shrinkage of the cells. When these authors treated the ganglia before fixation with hypertonic saline they found, on subsequent staining with osmic acid, that the number of dark-stained cells was increased, and this they considered was due to the greater shrinkage caused by the hypertonic saline. They further stress the "shrinkage space" around the dark-stained cells, and observe that cells with a smaller surrounding space are grey in colour, whereas the very pale cells show little or no "shrinkage space". The present writers, however, have observed that the "space" around the dark multangular cells is to a large extent occupied by subcapsular satellite cells which are not clearly stained by the osmic acid technique; it is therefore not entirely a shrinkage space. Kiss himself was careful to point out (1932) that shrunken cells are useless for this study; both colour and form, he observes, are influenced by shrinkage. The present authors do not deny that, even when following strictly the technique of Kiss, some

shrinkage of the dark cells may occur. But, as a rule, there is no evidence of shrinkage of the pale cells, and this suggests a greater sensitivity to the reagents on the part of the dark cells, which is probably due to their different physico-chemical constitution. In our preparations the exceptional pale cells which showed any sign of a shrinkage space around them still remained pale cells.

This suggestion of a physico-chemical difference between the pale and dark cells is confirmed by Bacsich (1932), who found that the dark cells combined with more osmium than the pale cells by virtue of their greater lipoid content, and by Blair and Davies (1933), who correlated Bacsich's finding of coarser protein-lipoid granules in the dark cells with the meshes of a coarser neuro-fibrillary network in these cells which is clearly revealed by infra-red photography.

In the present work the experiment of Fisher and Ranson, involving the treatment of the ganglia with hypertonic saline, was repeated. A number of spinal ganglia were removed from a young cat, and comparison was made between the specially treated ganglia of one side and the corresponding ganglia of the opposite side treated by the prolonged osmic acid method of Kiss. The ganglia to be specially treated were, immediately after removal, placed in hypertonic (3 per cent.) saline for 3 hours. They were then fixed in 10 per cent. formalin and subsequently treated by the osmic acid method. Examination after sectioning revealed an obvious increase in the number of dark-stained cells as compared with the opposite normally treated ganglia (Plate IV, fig. 18). Furthermore there were all grades of coloration, varying from very pale to very dark stained nerve cells, in contrast with the crisp difference between pale and dark cells of the normal preparation (Plate IV, figs. 18, 20). But a marked difference between the normal and the saline-treated ganglia was observed in respect of the Golgi reticulum. In the normal ganglia the Golgi reticulum, as above described, was well stained, and was of a coarser and denser nature in the dark cells than in the large and small pale cells. In none of the dark-stained cells of the saline preparations could any Golgi apparatus be made out (Plate IV, figs. 19, 20). This is evidence that these cells are damaged and, far from there being an increase in the number of *true* dark cells after saline treatment (as Fisher and Ranson described), we are of the opinion that none of the cells now staining darkly exhibit the characteristic appearances of normal dark cells: all the cells of the ganglia, pale and dark, have been damaged by the saline treatment. In other words, all the dark cells are altered cells, and it is now difficult if not actually impossible in most cases to determine which of the very dark-stained cells were originally true dark cells and which are pale cells, shrunken by the saline with consequent increase in concentration of lipoid content and darker staining reactions with the osmic acid. It is noteworthy too, that ghosts of the Golgi network are present only in the least shrunken and in the palest cells (Plate IV, fig. 19); some cells, although shrunken, were still pale. We therefore conclude that shrinkage by means of hypertonic saline may cause a cell to stain darker with osmic acid (in virtue

of the purely physical osmotic process of increase of the lipoid concentration), but such damage to the cell is revealed by the absence of the Golgi reticulum. Further, the presence of a well-defined Golgi network in the dark cells of spinal ganglia treated by the osmic acid method of Kiss, is evidence that these dark cells are healthy, and the osmic reaction indicates that these cells have a different chemical composition from that of the pale-stained cells.

That the multangular shape of the dark cells is not due solely to shrinkage is strongly suggested by the fact that, in the normal preparations, occasional pale cells are sometimes shrunken but still retain their rounded or oval form.

We tried the effect of prolonging the period of immersion of the ganglia in osmic acid from the 5–8 days recommended by Kiss to 20 days, in order to find out whether this longer treatment would darken the pale cells and thus increase the apparent number of dark cells. But this experiment revealed the same histological picture of dark cells in normal proportion and the pale cells (large and small) still retained their pale-stained features (Plate IV, fig. 21).

With regard to the question of whether the dark cells are the product of age changes in the ganglia, we observed that the dark cells are present in the spinal ganglia of the new-born kitten in proportion similar to that in adult material.

The question also arose whether the dark-stained cells represented a stage in the functional activity of the nerve cells. This appeared to us not to be the case, as the dark-stained cells are nearly all of the same size (intermediate between that of the large and small pale cells), whereas, if they represented a stage in the activity of the cells, dark cells of all sizes would be expected to be present. Further, the toluidin blue stain reveals the presence of coarse tigroid granules in the dark-stained multangular cells (Plate III, figs. 14–16). If the cells were in an exhausted stage, the condition would be expected to be one of tigrolysis.

There is none of the generally recognised features of cell injury or degeneration visible in the dark-stained cells (e.g. karyolysis, karyorrhexis, pyknosis, excentric position of the nucleus, tigrolysis), and taking into account all the histological features of these cells (e.g. good nuclear structure, central nuclear position, well-marked neurofibrillary network, well-defined Golgi reticulum, coarse tigroid bodies) we conclude that they represent a normal morphological type of healthy spinal ganglion cells. In this connection it may be pointed out that, although Cajal and his school believe that the occasional atypical (true multipolar) cells found by them in spinal ganglia are pathological, we have already stressed that multipolar nerve cells are much less numerous than the dark-staining cells described in the present paper.

As described above, the dark-stained multangular cells of the posterior root ganglia differ from the rounded pale cells in internal structure, containing coarser (lipoid) granules stained by osmic acid, coarser tigroid bodies stained by toluidin blue, denser neurofibrillary network revealed by silver impregnation, and denser Golgi network. In this connection it may be noted that several

observers, notably Cajal (1928 *a*, *b*), Jacobsohn (1910), Malone (1913 *a*, *b*), have attached importance to the relation between the internal structure of nerve cells and their function. Cajal, for instance, recognises a stichochrome type of nerve cell, characterised by its coarse tigroid granules, as being motor in function (e.g. motor cells of spinal cord and medulla oblongata, pyramidal cortical cells), and a gryochrome type, characterised by powdery tigroid granulation, such as that found in the majority of cells of the spinal ganglia, and having a sensory function. Other intermediate types also are recognised by this author. The dark cells of the spinal ganglia as described by us appear to belong to the stichochrome type (Plate III, fig. 16), but we do not at present wish to attach finally any particular functional significance to these dark cells.

Kiss based his functional interpretation of the dark cells in the spinal ganglia on their similar staining reactions with osmic acid to the dark cells found in much larger proportion in the sympathetic ganglia. We have examined for ourselves the question of the morphological relationship of the dark-stained cells in the spinal and sympathetic ganglia with a large variety of staining methods. We find that there is a close resemblance in the internal appearances of the cyton between the dark cells of the posterior root ganglia and those of the sympathetic ganglia, as revealed by the osmic acid method of Kiss, the toluidin blue method and various silver methods. But there is a distinct difference between these two kinds of cells in respect of cell processes. In the sympathetic ganglia the dark-stained cells are readily shown to be multipolar (having an axon and several dendritic processes) by the formalin-pyridine-silver method (Blair and Davies, 1935) (Plate III, fig. 17), and also by other silver methods which do not so well differentiate the dark and pale cells. In the spinal ganglia, on the other hand, most careful examination by a variety of silver techniques showed multipolar cells to be highly exceptional. The great majority are unipolar and a few bipolar. The similarity of internal structure of the dark cells of the spinal and sympathetic ganglia does suggest a possible similarity in function, and the almost complete absence of multipolarity of the dark cells of the spinal ganglia does not necessarily exclude this suggested functional relationship. The existence of dark-staining multangular cells in the posterior root ganglia (by the osmic acid technique, toluidin blue method, and various silver methods) is undoubted and we hope to study by experimental methods the possibility of their functional relationship to that of the dark-staining cells of the sympathetic ganglia.

It may be noted finally that the ordinary laboratory stains, like logwood and eosin, differentiate the large pale cells, the small pale cells and the multangular darker stained cells, though not nearly so crisply as the prolonged osmic acid method of Kiss.

SUMMARY AND CONCLUSIONS

1. The nerve cells of the spinal ganglia of the cat have been examined by many histological methods—prolonged osmic acid method, toluidin blue and various silver techniques.

2. All these methods differentiate three types of ganglion cells—large round pale, small round pale, and dark multangular cells.

3. Micro-incineration and the study of the Golgi apparatus also demonstrate the separate morphological identity of the dark cells.

4. The dark cells of the spinal ganglia differ from those of the sympathetic ganglia in being rarely multipolar.

5. Evidence is given that the dark cells of the spinal ganglia are not the product of shrinkage or degeneration. Their different appearance from that of the pale cells is presumably due to a different physico-chemical constitution which may be associated with a different function.

6. Certain internal structural features of the dark cells of the spinal ganglia are among those recognised by other workers as typical of motor nerve cells, but judgment on this point is reserved pending the outcome of experimental studies.

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We are greatly indebted to Dr E. S. Horning for his helpful co-operation in preparing sections of spinal ganglia for demonstration of the Golgi apparatus, and in applying the micro-incineration technique to our material. We have also to thank him for the photographs used for Plate II, figs. 6 and 7. We also wish to express our gratitude to our assistant, Mr S. Hogwood, for his help in the preparation of the photomicrographs.

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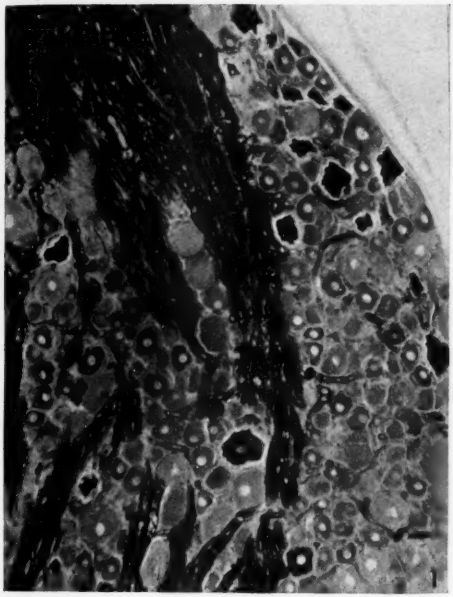
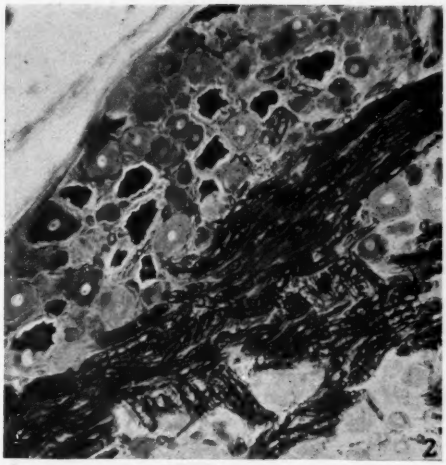
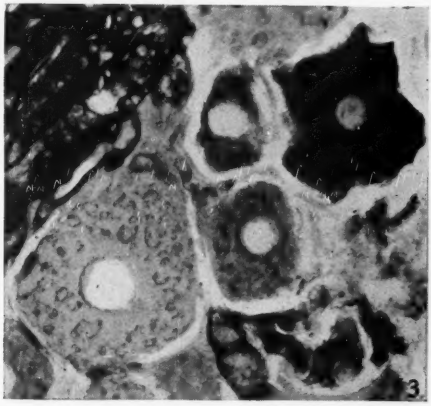
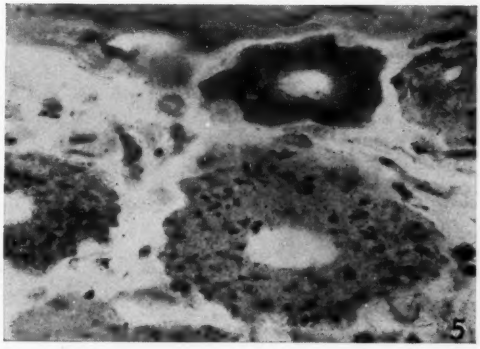
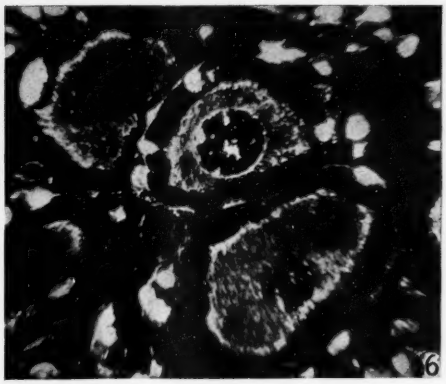


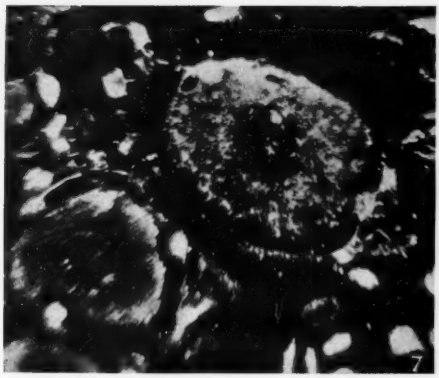


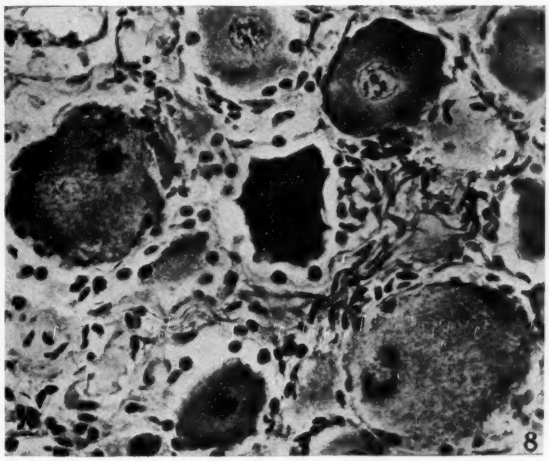


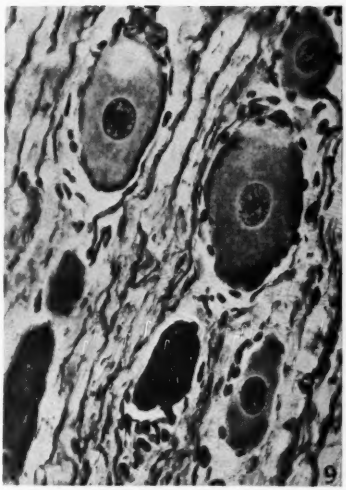


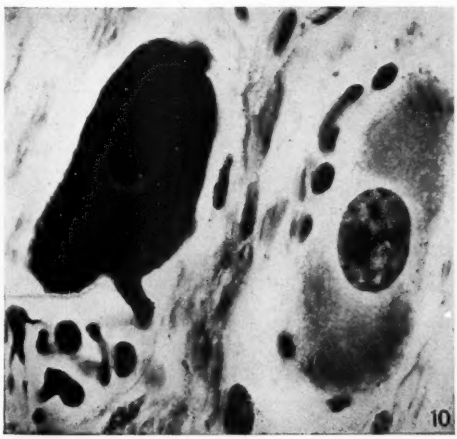
BLAIR, BACSICH AND DAVIES—THE NERVE CELLS IN THE SPINAL GANGLIA

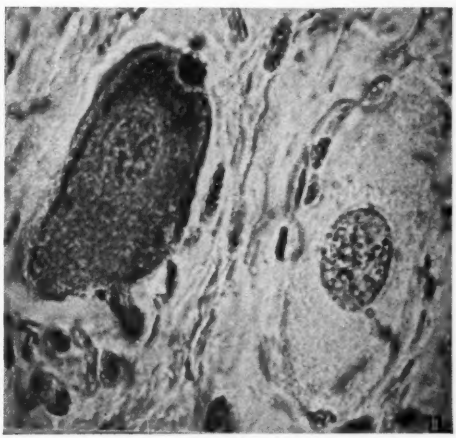












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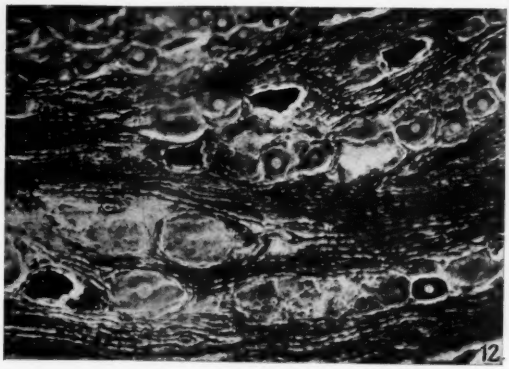

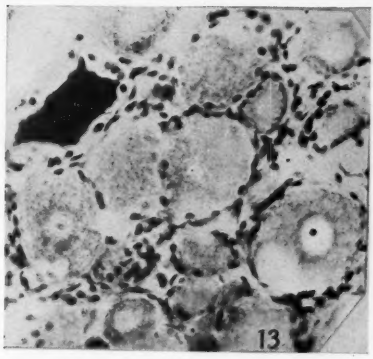

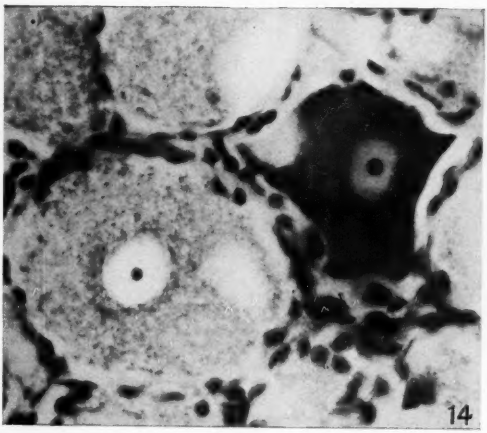

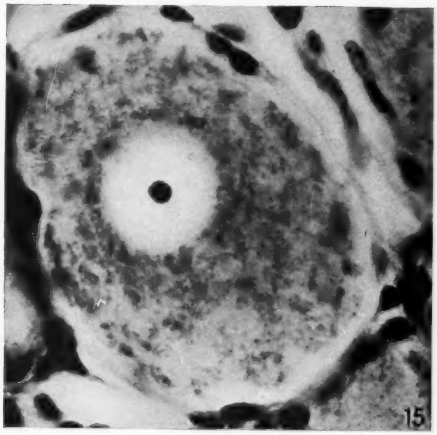

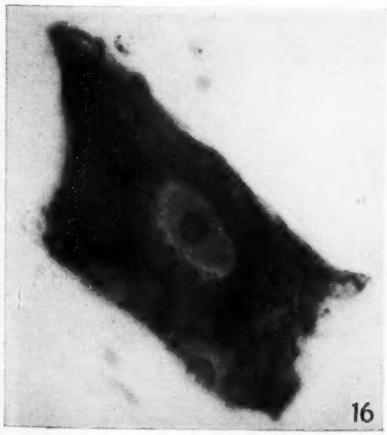

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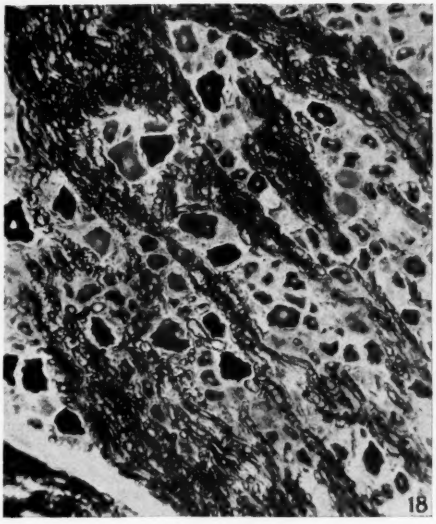

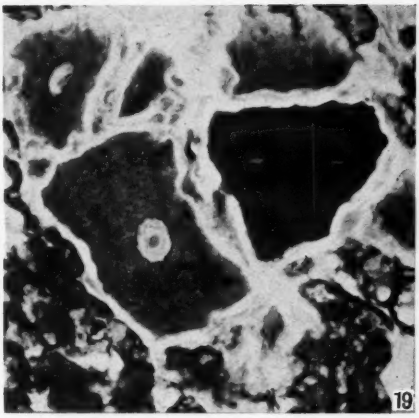

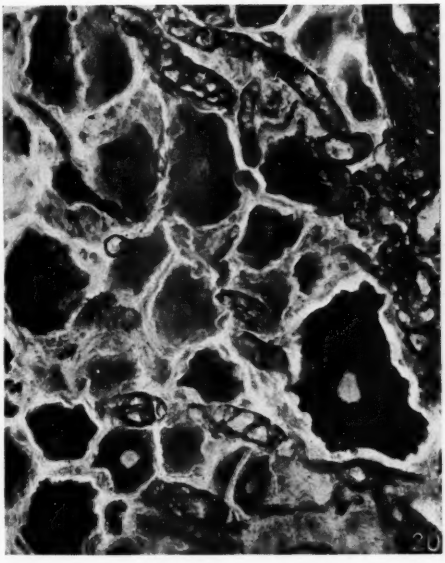

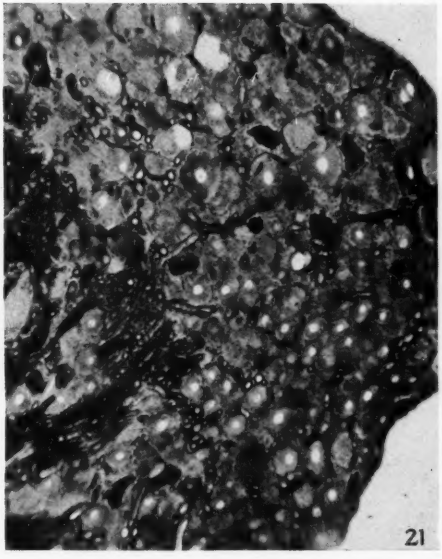

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EXPLANATION OF PLATES I–IV

(All figures are untouched photomicrographs)

PLATE I

Fig. 1. Spinal ganglion (cat). ×95. Osmic acid. Shows normal distribution of the three types of nerve cells described.

Fig. 2. Spinal ganglion (cat). ×110. Osmic acid. Same as fig. 1.

Fig. 3. Spinal ganglion (cat). ×485. Osmic acid. Shows the three types of nerve cells with well-marked Golgi reticulum.

Fig. 4. Spinal ganglion (cat). ×990. Osmic acid. High-power view of dark cell. Note coarse Golgi apparatus and nuclei of satellite cells.

Fig. 5. Spinal ganglion (cat). ×485. Da Fano's technique. Shows characteristically different appearance of the Golgi apparatus in the pale and dark cells.

PLATE II

Fig. 6. Spinal ganglion (cat). ×630. Micro-incinerated section prepared and photographed by Dr E. S. Horning. (Dark ground illumination.) Shows finely powdered mineral ash of cytoplasm in three small pale cells.

Fig. 7. Same as fig. 6. ×630. Shows more "lumpy" appearance of mineral ash of dark cell. Note characteristic indentations on lower edge of cell occupied by satellite cells.

Fig. 8. Spinal ganglion (cat). ×425. Silver impregnation (Blair and Davies). Shows differentiation of the three cell types.

Fig. 9. Same as fig. 8. ×315.

Fig. 10. Higher power view of dark and small pale cell seen in lower part of fig. 8. ×990. Panchromatic plate.

Fig. 11. Same as fig. 10. ×990. Infra-red plate. Note coarse neurofibrillary network revealed in dark cell.

PLATE III

Fig. 12. Spinal ganglion (cat). ×150. Cajal-De Castro silver technique, frozen section. Note good differentiation of all three cell types.

Fig. 13. Spinal ganglion (cat). ×245. Toluidin blue. Shows the three types of nerve cells.

Fig. 14. Same as fig. 13. ×425. Shows characteristic differences in tigroid content of dark and large pale cell.

Fig. 15. Same as fig. 13. ×850. Shows gryochrome type of tigroid content in large pale cell.

Fig. 16. Same as fig. 13. ×990. Shows stichochrome type of tigroid content in dark cell.

Fig. 17. Superior cervical sympathetic ganglion (cat). ×505. Silver impregnation (Blair and Davies). Shows small pale cells and true multipolar dark cells.

PLATE IV

Fig. 18. Spinal ganglion (cat). ×100. Osmic acid. Effect of hypertonic saline treatment. (See text.)

Fig. 19. High power view of portion of fig. 18. ×485. Note nuclear crenation and indefinite appearance of Golgi reticulum in pale cells.

Fig. 20. Same as fig. 18. ×360.

Fig. 21. Spinal ganglion (cat). ×100. Osmic acid 20 days. The three types of cells are still recognisable; no increase in number of dark cells. (Cf. Plate I, figs. 1, 2.)

THE INNERVATION OF THE KIDNEY, URETER, TESTICLE AND EPIDIDYMIS

By G. A. G. MITCHELL, M.B., Ch.M.
Assistant in Surgery, Aberdeen University

Within recent years our knowledge of the autonomic nervous system has been greatly increased but there is still much to be discovered. There is also a great deal to be forgotten—inaccurate anatomical observations, physiological experiments based on false anatomical conceptions, and unsound surgical procedures founded on inadequate knowledge of both anatomy and physiology. The seeker after truth in the literature on the autonomic nervous system has no easy task, and he is apt to be confused by conflicting statements. The difficulties of those who tackle the problem directly are equally great, for the complexity of the intercommunications within the system and the small size of many of the nerves combine to render direct anatomical study difficult, and lack of accurate anatomical knowledge renders physiological investigations of less value than they would otherwise be.

Operations on various portions of the autonomic nervous system are becoming increasingly common, and this has intensified the interest in sympathetic and parasympathetic nerves. Their anatomical arrangement and the possibility of variations has become of vital importance, and the value of anatomy as an approach to surgery has seldom been better illustrated than in this subject. The abdominal portion of the system has received most attention, because it is the largest, most intricate and most interesting part of the system, and because its various parts have become favourite objects of surgical attack. The operations have not always been successful, and as many of the failures have been largely due to inaccurate anatomical knowledge, it is hoped that the following description of the disposition of parts of the abdominal autonomic nervous system may have a certain practical value.

The observations are based on a series of fifteen dissections, which (owing to the difficulties in obtaining adult subjects) were performed on still-born babies, but as the plan of the autonomic nervous system never alters once it is fully differentiated, young children are suitable for an investigation of this type. The dissections were performed with ophthalmic instruments under a weak solution of spirit, and with the aid of a low-power binocular microscope. At first, various stains, dilute acetic acid, etc., were used in an attempt to facilitate dissection, but were soon abandoned as their use did not produce the desired results. Recently one discovered by accident a very simple method which not only facilitates dissection, but also permits the course of the auto-

nomic nerves to be traced with the minimum of dissection and disturbance. Moreover, it shows up readily delicate fibres which even the finest dissection would almost certainly destroy. It may be called the *water-infiltration* method, and it consists in holding the specimen for several minutes at a time under a stream of cold running water *after* careful removal of the peritoneum or pleura. A specimen with the minimum of adipose tissue should be selected for the purpose, and the stream of water should be in the form of a smooth column, as this avoids infiltration of the tissues with air bubbles; a moderate rate of flow is all that is necessary. The water permeates the areolar network of the extraperitoneal tissues and mesenteries, with the result that these areas become semi-translucent, and the nerves, blood vessels, and even the larger lymphatic vessels can be seen and traced with little difficulty. The process can be repeated frequently without destroying the specimen, and any areolar tissue which obscures the view is gently removed. In dissecting the abdominal autonomic plexuses freer access is obtained by removing the liver and small intestines, and by splitting the pubic symphysis, bladder, prostate, vesicles, and other tissues in the medium sagittal plane as far back as the rectum. The two halves of the pelvis are then forced apart so that the pubic bones are separated anteriorly by 1–2 in., and the parts are maintained in this position by any convenient method.

Photographic records were made of every dissection, and the negatives and prints have not been retouched in any way. Satisfactory results were difficult to obtain on account of the small size of many of the nerves and their resemblance in colour to surrounding parts, and many delicate nerve filaments which could be seen naked eye, or with the aid of a dissecting microscope, are not visible in the photographs. But the difficulties were not only technical. Considerations of time and expense were also important, for in order to secure a clear representation of every nerve, each dissection would have had to be photographed from numerous angles. Black paper placed behind the nerves rendered photographic reproduction much easier, but this time-consuming method was accompanied by a high "mortality" when dealing with the finer nerve strands. The pen-and-ink drawings, with the exception of the first three figures, were obtained by outlining the essential features on a matt-surface photograph with waterproof Indian ink, and then bleaching the print by chemical action, a method which ensured perfect accuracy. As a further check Prof. Learmonth, who instigated and directed the investigation, has confirmed every important finding by an examination of the actual dissections, and the writer is greatly indebted to him for his valuable help. In the earliest dissections the exact nature of several strands was in doubt, and these were examined microscopically to prove or disprove the presence of nerve fibres. Increasing experience, and the discovery of the "water-infiltration" method of dissection, have rendered microscopic confirmation unnecessary in later dissections.

Particular attention was devoted to the disposition of the nerves to the kidney, ureter, testicle and epididymis, and this necessitated widespread

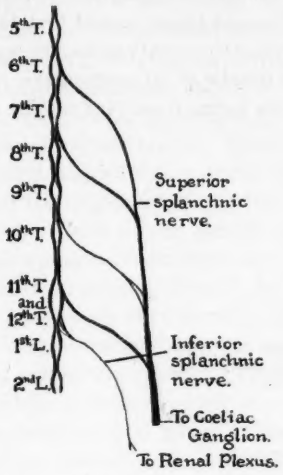
dissection of the parent nerves and plexuses. It will be convenient to describe the latter separately, before discussing the innervation of the individual viscera, but before doing so it is well to emphasise that the autonomic nerves are more variable in their arrangement than somatic nerves, a finding which may be explained by the highly developed migratory powers possessed by the sympathochromaffin cells from which the autonomic nerves and plexuses are developed. Moreover, the frequent plexus formations in the autonomic nervous system favour variations, for the same fibres have a choice of several alternative routes to reach most of the viscera, and, just as in the somatic system, certain nerve fibres may become associated for a longer or shorter course than usual. A high division of the great sciatic nerve does not mean that the muscles and cutaneous areas normally supplied by certain spinal segments receive their supply from any other sources, and mere macroscopic variations in the autonomic arrangement do not necessarily indicate that the ultimate source of the nerve fibres to any one organ are different. These important facts have not received the emphasis they deserve. Despite variations, however, a basic plan is always evident, and this will receive first attention in the following description.

The splanchnic nerves

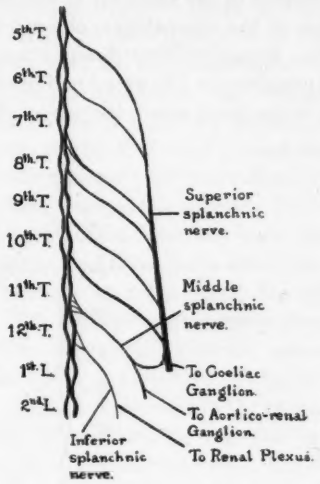
As a rule there are three splanchnic nerves on each side, and they arise from the thoracic portion of the sympathetic trunks. They are usually termed the greater or large, the lesser or small, and the lowest or least splanchnic nerves, but these names are unsatisfactory because the so-called lowest or least nerve is not always the smallest. To avoid confusion they would be better termed the superior, middle and inferior splanchnic nerves, or superior and inferior when only two are present.

(1) *The superior splanchnic nerve* is the largest of the three, and arises by a variable number of branches from the thoracic sympathetic trunk of the same side. As a rule there are three or four larger and an inconstant number of smaller roots. The highest root is often the largest both in regard to calibre and length, and it usually arises opposite the level of the fifth or sixth thoracic vertebra. In one dissection, however, it arose at the level of the third thoracic vertebra, and in another it arose opposite the eighth thoracic vertebra. The lowest root may arise as high as the eighth or as low as the tenth thoracic vertebra, but in one dissection where the middle splanchnic nerve was absent, the lowest root arose from fused eleventh and twelfth thoracic ganglia (text-fig. 1); presumably the lowest root represented the middle splanchnic nerve which, in this instance, had fused with the superior splanchnic nerve. In another specimen the superior splanchnic nerve was formed by six branches of equal size which arose from the fifth to the tenth thoracic ganglia inclusive, or from the intervening portions of the sympathetic chain (text-fig. 2): this is the mode of origin described and featured in many books and articles, but it is doubtful if it is the most common. The various roots pass obliquely forwards, inwards, and downwards across the sides of the thoracic vertebrae, lying

antero-external to the intercostal vessels, and immediately adjacent to the parietal pleura. They unite to form a nerve of considerable size which pierces the crus of the diaphragm and ends directly in the outer part of the semilunar ganglion. The nerve becomes slightly broader and flattened and occasionally it divides into two or more divisions before entering the ganglion, while it always gives off several delicate branches to the suprarenal body which lies immediately antero-external to its termination. The superior and middle splanchnic nerves communicate by a branch of variable size either before or



Text-fig. 1.



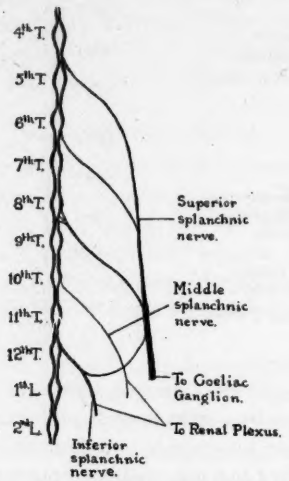
Text-fig. 2.

after they pierce the diaphragm. In one specimen the superior and inferior splanchnics communicated by a cross branch, and the middle splanchnic nerve, which was small and arose by a single root from the tenth thoracic ganglion, communicated with neither but ran, without branching, into the renal plexus (text-fig. 3). In this series of dissections no ganglion splanchnicum (Lobstein-Arnold) has been noted at any point along the course of the superior splanchnic nerve.

(2) *The middle splanchnic nerve* is usually intermediate in size between the superior and inferior splanchnics. It is formed by the fusion of one or more rootlets which arise from the thoracic sympathetic chain opposite the last two or three thoracic vertebrae. These pass forwards, inwards and downwards and unite to form a single small nerve which pierces the crus of the diaphragm just external to the superior splanchnic nerve, and ends in the renal plexus or in the aortico-renal ganglion. It communicates either above or below the diaphragm with the superior splanchnic nerve, and less frequently it is united by a cross branch or branches with the inferior splanchnic nerve near the origin of the renal plexus. In one dissection the middle splanchnic nerve was absent,

and was probably represented by the lowest root of the superior splanchnic nerve which arose from fused eleventh and twelfth thoracic ganglia (text-fig. 1). In another case the middle splanchnic nerve was much smaller than either the superior or inferior splanchnic nerves, did not communicate directly with them, and ended in the renal plexus (text-fig. 3). In three dissections the left middle splanchnic nerve passed behind the vena hemiazygos, instead of lying, as usual, between the vein and the parietal pleura.

(3) *The inferior splanchnic nerve* is not constantly present, and is usually the smallest of the three. It arises from the last thoracic ganglion, or from the portion of the sympathetic chain between the last thoracic and first lumbar ganglia. It passes forwards and inwards and joins the renal plexus, the aortico-renal ganglion, or the upper end of the outer bundle of intermesenteric nerves close to the point where they give off filaments to the renal plexus. The nerve



Text-fig. 3. In the specimen the portion of the lateral sympathetic chain between the last thoracic and first lumbar ganglia consisted of three main fibres with oblique anastomosis; this arrangement is not shown in the diagram.

enters the abdomen by accompanying the lateral sympathetic chain beneath the medial lumbo-costal arch. In two dissections this nerve was larger than the middle splanchnic nerve, in one case being distinctly so. In this latter case the nerve arose by a single root from the last thoracic ganglion and ended in the renal plexus, but before doing so it was united to the superior splanchnic nerve by a branch of considerable size (text-fig. 3). In this specimen the portion of the lateral sympathetic chain between the last thoracic and first lumbar ganglia consisted of three main fibres with oblique anastomoses instead of the more usual single nerve. This plexiform arrangement of the lateral sympathetic chain between the last thoracic and first lumbar ganglia has been noted in other

cases. In one specimen where there was no separate left inferior splanchnic nerve two rami arose from the first left lumbar ganglion; one of these joined the intermesenteric nerves and the other joined the renal plexus; the latter was probably the inferior splanchnic coming off the sympathetic trunk at a slightly lower level than normal.

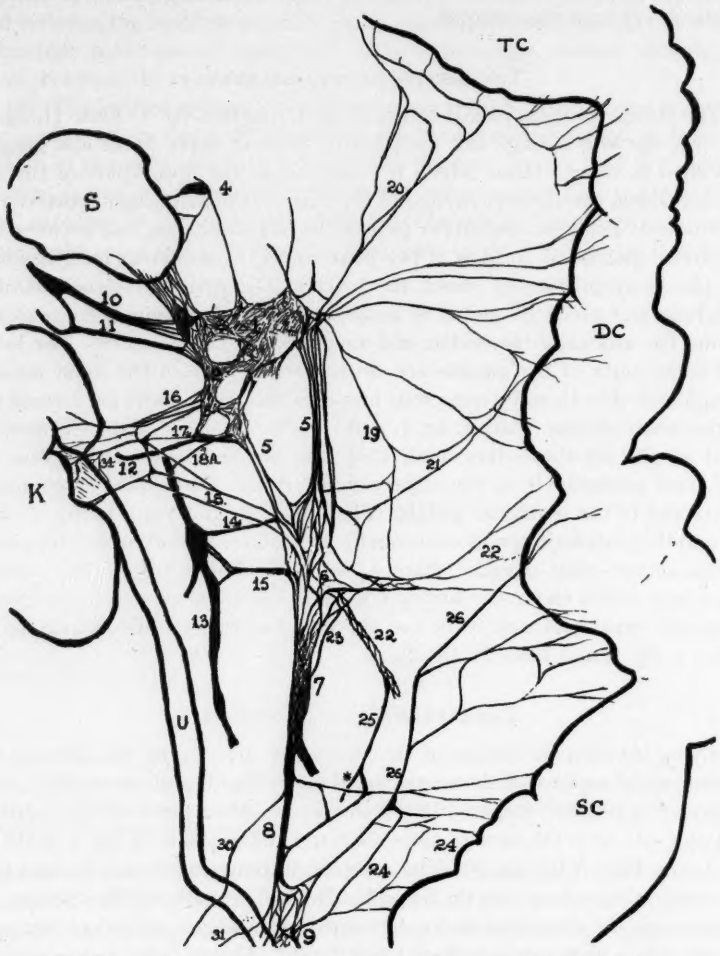
The coeliac or solar plexus

The coeliac or solar plexus (Plate I, fig. 1, and text-fig. 4; Plate II, fig. 2, and text-fig. 5) is a large and complicated mass of nerve fibres and ganglia imbedded in fibrous tissue which lies anterior to the upper part of the abdominal aorta, the cisterna chyli, and the crura of the diaphragm, posterior to the stomach, pancreas, and upper part of the omental bursa, and between the suprarenal glands. It consists of two large coeliac or semilunar ganglia which are placed symmetrically about the level of the first and second lumbar vertebrae, and which are united by numerous filaments forming a dense plexus around the origins of the coeliac and superior mesenteric arteries. The lower and outer parts of the ganglia are usually separate from the main masses, though united to them by numerous branches, and these parts are termed the aortico-renal ganglia (Plate I, fig. 1, and text-fig. 4): occasionally the aortico-renal ganglia are themselves subdivided. The plexus receives twigs from the vagi, and probably from the right phrenic nerve. The superior splanchnic nerves end in the semilunar ganglia (Plate I, fig. 1, and Plate II, fig. 2), and the middle splanchnic nerves occasionally do so, but more often they terminate in the aortico-renal ganglia (Plate I, fig. 1, and text-fig. 4). The inferior splanchnic nerves end in the aortico-renal portions of the plexus, in the closely associated renal plexuses, or in the upper end of the intermesenteric nerves (Plate I, fig. 1, and Plate II, fig. 2).

The intermesenteric nerves

Lying between the origins of the mesenteric arteries, on the anterior and antero-lateral aspects of the aorta, are delicate bundles of nerve fibres, four to twelve in number, disposed in the shape of a closed fan with the narrower end upwards, or in the form of more discrete bundles (Plate II, fig. 2, Plate IV, fig. 4, and Plate VIII, fig. 10). These are the intermesenteric nerves, and they are nearly always larger on the left side. Not infrequently, as Hovelacque has emphasised, the outermost nerves lie lateral to the aorta and do not rest upon it, and this is seen more often on the left side. Above, these nerves are continuous with the coeliac plexus. The outer fibres are usually united to the lower parts of the semilunar or aortico-renal ganglia, and the intermediate fibres join the cross branches between the semilunar ganglia, particularly those which lie below the origin of the superior mesenteric artery. Below, some fibres form a plexus around the origin of the inferior mesenteric artery and others pass directly downwards into the superior hypogastric plexus. They

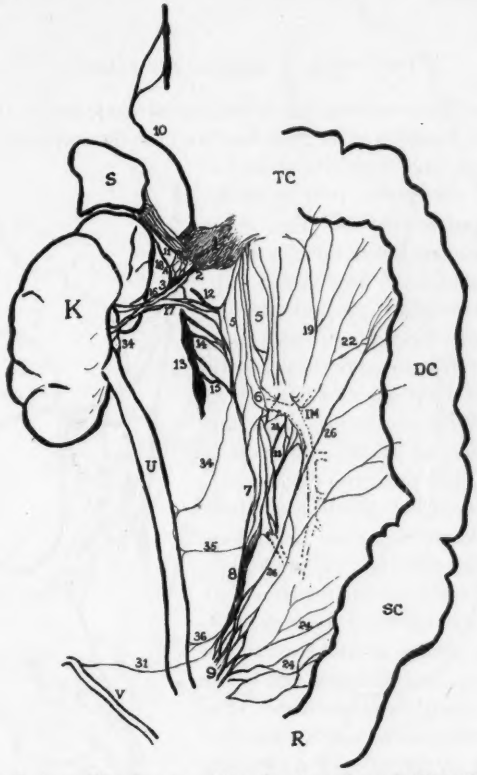
anastomose by several fine oblique branches which are more frequent near their termination, but a true plexiform arrangement has been seen only in one specimen—curiously enough the last to be dissected in this investigation.



Text-fig. 4. An outline drawing of the dissection shown in Plate I, fig. 1.

Opposite the hilum of each kidney they give off two to five slender branches which pass to the aortico-renal ganglia or directly to the renal plexuses (Plate I, fig. 1, and Plate II, fig. 2). In most cases an additional one or two delicate twigs pass upwards and outwards from the lower and outer parts of the inter-mesenteric nerve group to take part in the formation of the renal plexuses:

at their lower ends these nerves communicate with the inferior mesenteric plexus, and they appear to be more constant on the right side (Plate I, fig. 1, and text-fig. 4). The intermesenteric nerves often give off one or more small branches which accompany the spermatic vessels, and they are joined laterally by two to four substantial rami from the upper half of each lumbar sympathetic chain. The upper ramus (or rami) joins the intermesenteric nerves directly



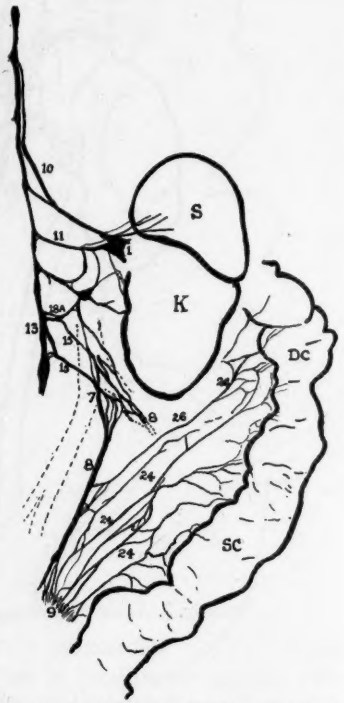
Text-fig. 5. An outline drawing of the dissection shown in Plate II, fig. 2

(Plate II, fig. 2, and text-fig. 5). The lower two not uncommonly fuse before they join the termination of the intermesenteric nerves or the beginning of the superior hypogastric plexus (Plate I, fig. 1, and Plate II, fig. 2). These rami are often termed the lumbar splanchnic nerves and they cannot be demonstrated clearly on the right side until the inferior vena cava is removed. In one dissection a curious loop was seen which united the left outermost intermesenteric nerve to the plexus around the inferior mesenteric artery about $\frac{1}{2}$ in. from its origin (Plate IV, fig. 4, and text-fig. 7). Almost invariably a nerve loop is seen lying along the inner margin of the inferior mesenteric vein, uniting

the upper part of the left intermesenteric nerves, or that part of the coeliac plexus which surrounds the origin of the superior mesenteric artery, to the inferior mesenteric plexus. The intermesenteric nerves give off numerous fine branches which unite with branches from nearby nerves and plexuses and form an extensive open-meshed network in the retroperitoneal tissues. These delicate fibres are particularly well shown up by the use of the "water-infiltration" method.

The inferior mesenteric plexus

The plexus which surrounds the inferior mesenteric artery (Plate IV, fig. 4, and text-fig. 7) is constituted by branches from the intermesenteric and lumbar splanchnic nerves, and below its fibres are continuous with the central portion of the superior hypogastric plexus. The outer bundles of intermesenteric nerves, in which the lumbar splanchnic nerves end, apparently take little part in the formation of the inferior mesenteric plexus and pass downwards into the superior hypogastric plexus. A nerve loop lies along the inner side of the inferior mesenteric vein and unites the coeliac or superior mesenteric plexus above to the lower end of the intermesenteric nerves or the inferior mesenteric plexus below. Another curious nerve loop sometimes unites the inferior mesenteric plexus to the intermesenteric nerves (Plate IV, fig. 4, and text-fig. 7). Its lower end is attached to the plexus around the inferior mesenteric artery, and its upper end to the left outermost intermesenteric nerve. The most convex portion of the loop is connected with the origin of the inferior mesenteric plexus by a fine nerve strand. This loop gives off several very fine branches (not seen in the photograph) which pass to the right and unite with filaments from the renal and spermatic plexuses. Occasionally small reddish nodules are detected amongst the fibres of the inferior mesenteric plexus and these may be termed the inferior mesenteric ganglia. A single large ganglion within the plexus has not been observed.



Text-fig. 6. An outline drawing of the dissection shown in Plate III, fig. 3.

THE SUPERIOR HYPOGASTRIC PLEXUS

The superior hypogastric plexus or pre-sacral nerve (Plate I, fig. 1, Plate II, fig. 2, Plate IV, fig. 4, etc.) is situated anterior to the bifurcation of the aorta, the left common iliac vein, the last lumbar vertebra, the middle sacral artery, and the lumbosacral intervertebral disc, and not in front of the sacrum as the

Text-fig. 7. An outline drawing of the dissection shown in Plate IV, fig, 4.

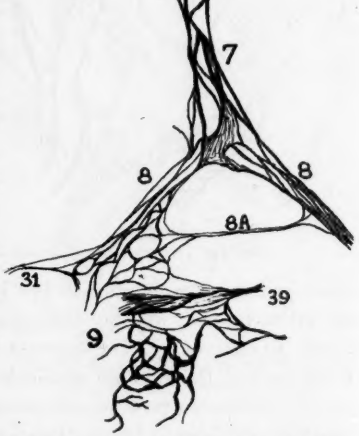
name pre-sacral suggests. It lies immediately beneath the peritoneum and can often be seen shining through this membrane, yet they do not adhere closely to each other. The superior haemorrhoidal vessels and the upper part of the root of the sigmoid mesocolon lie to its left side. The plexus is seldom exactly median in position, deviating a little to the right or more often to the left of the mid-line. As mentioned above the outer bundles of intermesenteric nerves apparently take little part in the formation of the inferior mesenteric plexus and pass downwards into the superior hypogastric plexus; the central parts of these two plexuses are connected by a few delicate fibres which lie in

front of the aorta. The superior hypogastric plexus generally receives one or two rami from the lower part of the lumbar sympathetic chain (Plate III, fig. 3, and text-fig. 6), and these pass forwards, inwards, and slightly downwards; lying either anterior or posterior to the common iliac artery. On the left side the superior hypogastric plexus invariably gives off branches which pass obliquely upwards and outwards to join the inferior mesenteric plexus not far from its origin (Plate I, fig. 1, Plate II, fig. 2, and Plate IV, fig. 4).

The plexus usually consists of a vertical flattened band of intercommunicating nerve bundles, wider below than above. There are usually two or three main bundles with smaller oblique anastomotic fibres. A single solid nerve is uncommon, being seen in only one of the fifteen specimens in this investigation, although the constituent parts of the plexus are enveloped in a condensation of the retroperitoneal areolar tissues which confer on it a false appearance of solidity. As a rule the constituent fibres of the plexus are not widely separated, except in a few cases, when they may be found straggling downwards across the whole inter-iliac trigone, that is, the triangular interval between the two diverging common iliac arteries and the sacral promontory. Below the superior hypogastric plexus broadens out and divides into the two hypogastric nerves, which pass downwards into the pelvis and end in the inferior hypogastric plexuses.

The hypogastric nerves

The hypogastric nerves are formed by the bifurcation of the superior hypogastric plexus (Plate IV, fig. 4, and Plate V, fig. 5). They diverge and pass outwards, downwards, and slightly backwards into the pelvis, lying some distance internal but almost parallel to the hypogastric arteries. Not infrequently the angle between the diverging hypogastric nerves is bridged across by several delicate interlacing fibres which form a network in front of the upper part of the sacrum (Plate V, fig. 5, and text-fig. 8); Davis terms this the middle hypogastric plexus. The hypogastric nerves may be solid (Plate IV, fig. 4, and Plate V, fig. 6), or may consist of two or more parallel bundles with oblique anastomosing strands forming an elongated plexus (Plate V, fig. 5, and Plate VI, fig. 7). Numerous branches are supplied by these nerves to the colon, testicle, ureter, etc., but these will be mentioned later under the appropriate headings. Branches are also supplied to the large blood vessels which lie in their vicinity



Text-fig. 8. An outline drawing of the dissection shown in Plate V, fig. 5.

and these are very fine and usually show distinct gangliform enlargements. Often five or six very slender filaments arise from the hypogastric nerve and pass to a small ganglion, and from the other side of the latter five or six equally slender filaments pass to the blood vessels. The appearance closely resembles the conventional drawing of a spider, and as many as three of these have been seen on one side.

The inferior hypogastric or pelvic plexuses

The inferior hypogastric or pelvic plexuses are situated on each side of the rectum, prostate, seminal vesicles, and the posterior part of the lateral wall of the bladder. They are large fenestrated sheets (Plate V, fig. 5, and text-fig. 8) arranged in a curved plane, the upper part being parallel to the lateral pelvic wall and the lower to the pelvic floor. Laterally each plexus is in relationship with the branches and tributaries of the corresponding hypogastric artery and vein, with the levator ani, and with small areas of the obturator internus, pyriformis and coccygeus muscles, the muscles being separated from the plexus by the above-mentioned vessels, by the parietal pelvic fascia, and by a variable amount of fatty tissue. The sacro-coccygeal plexus and its branches lie posteriorly, and the ureter crosses the superior border of the plexus from without inwards, or even appears to pierce the upper part of the plexus. At its termination the ureter is related to the inner surface of the anterior part of the plexus. The obliterated umbilical artery runs along its superior border, and the inferior border is in contact with the pelvic floor. Several minute vessels pass through fenestrations in the plexus. It is said that in adults a very distinct layer of parietal pelvic fascia intervenes between the plexus and the large veins and arteries on its outer side; this layer is difficult to distinguish in infants.

Each plexus consists of an intricate meshwork of nerve fibres and ganglion cells, embedded in firm connective tissue, and united to the adjacent rectum and bladder by numerous branches. The constituent nerves vary considerably in size, and their numerous anastomoses render the accurate tracing of individual nerve bundles almost impossible. The main connections are with the hypogastric nerves above, which join the postero-superior angles of the plexuses, and with the second, third and fourth sacral nerves behind through the pelvic splanchnic or visceral nerves (nervi erigentes). The pelvic splanchnics usually consist of from four to ten small nerves (Plate V, fig. 6), but sometimes one or two larger nerves are present (Plate V, fig. 5, and text-fig. 8). Each plexus also receives delicate filaments from the sacral portion of the sympathetic chain which enter its posterior border. These may arise from any of the sacral ganglia or from the intervening trunk. The most constant twig arises from the third sacral ganglion or the adjacent portion of the sympathetic chain.

According to many writers the inferior hypogastric plexus is distinctly bilaminar, consisting of an outer layer which is continuous with the pelvic splanchnic nerves, and an inner layer which is formed by a spreading out of

the corresponding hypogastric nerve. It is probable that this division is artificial, and there is no clear evidence, at any rate in infant subjects, that the plexus is bilaminar.

The nerves to the kidney

The nerves to the kidney accompany the renal vessels, and they arise from the following sources:

(1) From the aortico-renal ganglion.
(2) From the middle and inferior splanchnic nerves.
(3) From the intermesenteric nerves.
(4) From the lumbar sympathetic chain.

(1) Three to eight branches pass from the aortico-renal ganglion to the kidney, and near their commencement they may show a ganglion which is sometimes termed the renal ganglion (Plate I, fig. 1, and text-fig. 4). It must be emphasised, however, that ganglia are sometimes seen on the renal nerves which come from other sources. These nerves lie in contact with the renal artery, uniting with the other nerves which supply the kidney, and being joined together by transverse and oblique fibres which encircle the artery either partially or completely. In one specimen, after the renal artery was removed, two main trunks were seen connected by five transverse branches at regular intervals forming a perfect ladder pattern. The majority of the aortico-renal fibres pass along the superior border of the renal artery, and the upper branches often supply filaments to the suprarenal gland. These nerves are supposed to be constituted by fibres from the superior and middle splanchnic nerves, by filaments from the vagi, and by twigs from the opposite renal plexus which cross by way of the coeliac plexus. The first of these suppositions is accurate in certain cases at least, but it is difficult to confirm or disprove the others macroscopically.

(2) The middle splanchnic nerve occasionally ends directly in the renal plexus, but in other cases it terminates in the aortico-renal ganglion (Plate I, fig. 1, and text-fig. 4). The inferior splanchnic nerve often runs directly into the posterior part of the renal plexus (Plate I, fig. 1, and text-fig. 4), but it too may join the aortico-renal ganglion; rarely it joins the intermesenteric nerves close to the point where they give off branches to the renal plexus (Plate II, fig. 2, and text-fig. 5). Since both these nerves sometimes join the renal plexus directly it is clear that they must be intimately concerned with the renal innervation, and even when they join the aortico-renal ganglion or the intermesenteric nerves it is probable that the majority of their fibres are destined for the supply of the kidney. These nerves enter the posterior part of the renal plexus and are united by cross branches with its other constituents.

(3) The branches from the intermesenteric nerves may arise at two different points:

(*a*) They may arise at the level of the kidney hilum and pass almost directly outwards to the renal plexus (Plate I, fig. 1, and Plate II, fig. 2). The

maximum number of renal nerves which has been observed arising in this region is five but two or three is the commonest number. The greater the number the smaller the size and *vice versa*.

(*b*) One or two fine nerves may arise from the lower part of the outer bundles of intermesenteric nerves, and pass upwards and outwards to take part in formation of the renal plexuses (Plate I, fig. 1, and text-fig. 4). These are more often discovered on the right side. They may subdivide as they approach the kidney, and they lie along the inferior margin of the renal artery where they help to form the renal plexus: they show a particular tendency to pursue an independent course and their lowest fibre or fibres may be separated by a distinct interval from the other renal nerves, although they always communicate with the latter by one or more filaments. At their lower end they may communicate with the beginning of the inferior mesenteric plexus, but this is apparently not a constant occurrence, for in other cases it seems that they communicate only with those intermesenteric nerves which pass downwards into the superior hypogastric plexus. These renal nerves often communicate directly with the highest spermatic nerves, and in other cases they communicate indirectly through the delicate nerve network in the retroperitoneal tissues which, as already described, is formed by slender filaments from all the nearby autonomic nerves.

(4) The branch or branches to the renal plexus from the lumbar sympathetic chain is the least constant supply of the four (Plate I, fig. 1, and Plate II, fig. 2). It arises from the upper part of the lumbar chain, either from the first or second lumbar ganglion or from the intervening trunk, and it joins the posterior part of the renal plexus close to the termination of the inferior splanchnic nerve, uniting with this nerve and with renal branches from the intermesenteric nerve. At other times a lumbar ramus joins the intermesenteric nerves close to the point of origin of the renal branches, and it is possible that certain of the fibres in these renal nerves come from this lumbar ramus.

The renal plexus, therefore, is a composite structure formed by branches from widely separated sources, and this is a common feature in visceral innervation. This is the probable explanation of the experimental finding that denervation (so-called) of any particular viscus often produces surprisingly different effects in the hands of different workers. If one thing is certain it is this, that *complete* denervation of any viscus is difficult to obtain; for example stripping of the renal artery by no means ensures complete renal denervation, as the lower fibres from the intermesenteric nerves may remain untouched by this procedure, and these may exercise control over a sufficient amount of kidney tissue to mask any changes produced by the partial denervation.

The nerve supply of the ureter

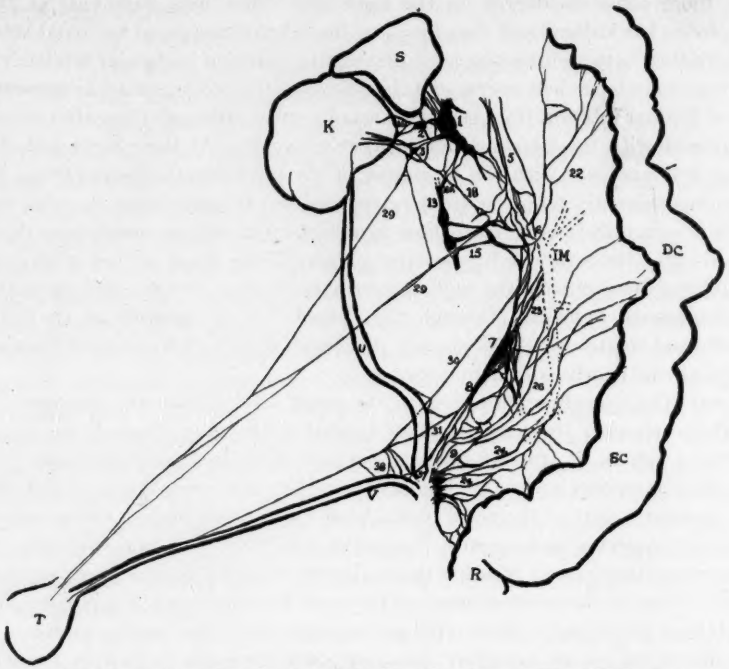
The ureter receives nerve filaments from three distinct sources:

(1) *A superior group* from the lower fibres of the renal plexus or from the intermesenteric nerves.

(2) *An intermediate or middle group* from the superior hypogastric plexus or from the upper end of the hypogastric nerve.

(3) *An inferior group* from the lower end of the hypogastric nerve and upper part of the inferior hypogastric plexus.

(1) *The superior ureteral nerves.* The lower fibres of the renal plexus give off several delicate twigs which run downwards along the upper part of the



Text-fig. 9. An outline drawing of the dissection shown in Plate VI, fig. 8.

ureter and supply it (text-figs. 4 and 11). This supply may be augmented by a direct branch from the intermesenteric nerves, or by a filament from those renal branches of the intermesenteric nerves which pass upwards and outwards from their lower ends. An inconstant twig may unite these ureteral nerves to the spermatic nerves.

(2) *The intermediate or middle ureteral nerves.* The intermediate portion of the ureter is supplied by one or two nerves which arise from the superior hypogastric plexus, or from the upper end of the corresponding hypogastric nerve (Plate IV, fig. 4, and text-fig. 7). When two are present the first branch arises from the side of the superior hypogastric plexus and the second arises at a lower level, or from the beginning of the hypogastric nerve. This nerve(s) often arises near to, or actually in conjunction with, one of the spermatic nerves, and its position is almost invariably indicated by a small ureteral artery which

arises from the common iliac artery close to its bifurcation, or from the commencement of the hypogastric artery; the branches of the artery and of the nerve interdigitate. In three specimens a different arrangement was found. A fine nerve detached itself from the side of the superior hypogastric plexus, passed downwards and outwards towards the ureter, and supplied it with several branches about the level of the first or second sacral vertebra. A part of the nerve, however, continued onwards to the outer side of the ureter and united with the main nerve to the vas deferens, which arose from the hypogastric nerve just before the latter terminated in the inferior hypogastric plexus. From the point of junction several filaments passed to the vas, and one at least to the lower end of the ureter. The common nerve trunk formed by the junction of the two nerves followed the course of the vas deferens and supplied it and the epididymis. This rather complicated arrangement can be readily appreciated by a study of the accompanying photograph (Plate VI, fig. 8). A somewhat similar arrangement is seen in Plate IV, fig. 4, but in this specimen there is a second ureteral nerve at a slightly lower level. Although an arrangement similar to the above was found only in three out of fifteen dissections, it is probable that this is an underestimation of its incidence, for these fine nerves are readily overlooked and easily broken. Unfortunately, this is true of most of the autonomic nerves, and thus any figures given under-estimate rather than over-estimate the incidence of any particular arrangement.

(3) *The inferior ureteral nerves.* The nerve supply for the lower end of the ureter is derived from the termination of the hypogastric nerve, from the upper part of the inferior hypogastric plexus, or from both (Plate II, fig. 2, Plate IV, fig. 4, and Plate VI, fig. 7). This part of the ureter is surrounded by a loop (text-fig. 10) which is formed by branches from the inferior hypogastric plexus, or by a branch from the hypogastric nerve which crosses the ureter to its outer side and then descends to unite in front of the ureter with a branch of similar size which arises from the upper part of the inferior hypogastric plexus. These ureteric nerves are closely associated with several filaments which supply the vas deferens and the seminal vesicle.

The nerves to the testicle and epididymis

As a rule the testicle and epididymis receive nerves from three different sources:

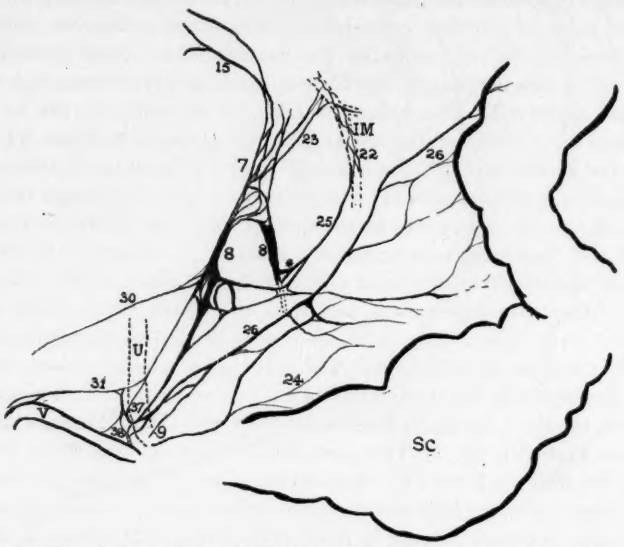
(1) *A superior group* from the intermesenteric nerves and from the renal plexus.

(2) *An intermediate or middle group* from the superior hypogastric plexus or from the upper end of the hypogastric nerve.

(3) *An inferior group* from the inferior hypogastric plexus.

(1) *The superior spermatic group.* There are usually two and sometimes three nerves in the superior spermatic group (Plate VI, fig. 8, and Plate VIII, fig. 10). The outer nerve arises by a few rootlets from about the middle and lower parts of the renal plexus (Plate VI, fig. 8, and text-fig. 9), but occasionally

it originates near the beginning or end of the plexus. The rootlets soon coalesce to form a single nerve (in two dissections two nerves were present) which converges towards the spermatic artery. It occasionally splits into two or three branches as it passes downwards, and it meets the spermatic artery at a variable distance from its origin, depending on the level of origin of this vessel from the abdominal aorta—the lower the level the further the nerve has to travel before establishing contact with it. This nerve lies to the outer side of the artery, communicates by several fine branches with the other superior



Text-fig. 10. The photograph from which this outline drawing was made is not reproduced.

spermatic nerve which arises from the intermesenteric group, and enters the postero-superior border of the testicle.

In most of the dissections a vein is seen arising from the outer side of the spermatic vein a short distance below the lower pole of the kidney (Plate VIII, fig. 10, and text-fig. 12). It passes upwards and outwards along the postero-external aspect of the kidney and receives tributaries from the peri-renal fatty tissue, from the veins of the posterior abdominal wall, and from the veins on the under aspect of the diaphragm. Nerve fibres connected with the superior spermatic nerves can be traced alongside this vein almost as far as the diaphragm, and they may actually establish communication with the splanchnic nerves or with the phrenic ganglion, although macroscopic connection could not be demonstrated. If this pathway really exists testicular afferents or efferents might conceivably utilise it as a direct or alternative route upwards or downwards from the splanchnic nerves, phrenic ganglion, etc. Be that as it may, afferent impulses from the testicle undoubtedly enter the cord at a

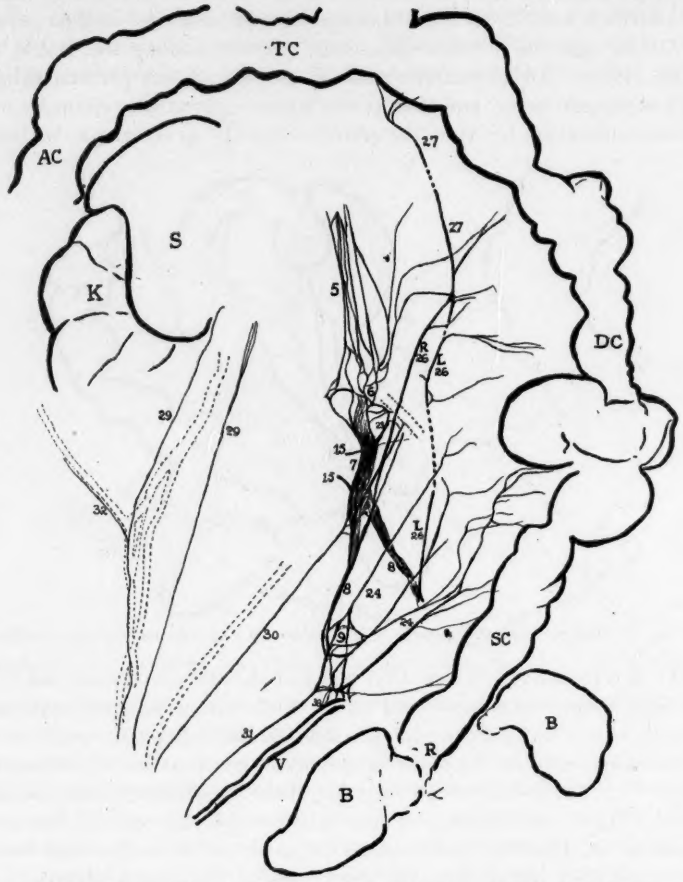
higher level than is commonly stated. An observation made by Learmonth on a patient under the influence of a spinal anaesthetic is the justification for this statement. The man, though completely anaesthetic as high as the level supplied by his ninth thoracic spinal segment, still retained normal testicular sensibility. Testicular afferents must have entered the cord, in this instance at least, above the level of the ninth thoracic segment, and they may have passed upwards through the superior splanchnic nerves or along the lateral sympathetic chains. An alternative pathway is through the phrenic ganglion, along the phrenic nerve, and then to the lateral sympathetic chain by one of the communications between the phrenic and the sympathetic within the

Text-fig. 11. The photograph from which this outline drawing was made is not reproduced.

thorax. It is unlikely, however, that many of the afferents would pass by this route, and it must be remembered that a direct communication between the spermatic nerves and phrenic ganglion was not demonstrated.

The inner nerve of the superior spermatic group arises by two or three rootlets from the lower end of the outer bundles of intermesenteric nerves (Plate VI, fig. 8, and text-fig. 9), but in a few cases several rootlets arise at a higher level. In other cases rootlets are given off from the renal branches which sometimes spring from the lower ends of the intermesenteric nerves. On the right side the rootlets of origin pass anterior to the inferior vena cava and on both sides they soon coalesce to form one or two larger nerves, but before doing so they often show gangliform enlargements; in one specimen (Plate IV, fig. 4, and text-fig. 7) three of these were present on the right side and two on the left. As a rule only one larger nerve is formed, and it approaches the spermatic artery and lies along its inner side. Before it has proceeded far if often subdivides into an outer smaller and an inner larger branch. The former adheres to the artery closely and may supply it. The latter is more easily

separated from the artery and communicates by several fine filaments with the spermatic nerve(s) which arises from the renal plexus; no true spermatic plexus formation was seen in any specimen. It usually maintains its position along the inner side of the spermatic artery and enters the testicle by several



Text-fig. 12. An outline drawing of the dissection shown in Plate VIII, fig. 10.

branches along the upper part of its posterior border. When two nerves are present one lies along the inner side of the artery and the other may lie anterior or posterior to it. These spermatic nerves did not wind around the spermatic vessel in a spiral fashion in any case investigated in this series of dissections.

The superior spermatic nerves are interesting for several reasons. They do not communicate macroscopically with the two other groups of nerves passing to the testicle and epididymis, they appear to be the only nerves which

enter the testicle directly, and they are closely associated with the renal and intermesenteric nerves. In one dissection (text-fig. 11) the right inner spermatic nerve arose from the outermost intermesenteric nerve on the right side at a higher level than usual, and the latter nerve divided immediately above, one branch passing upwards to the central portion of the coeliac plexus close to the superior mesenteric artery, the other being distributed to the third part of the duodenum. This was an interesting link between the testicular and intestinal nerves, and such a linkage may explain the sickness associated with all but the slightest testicular injuries. The fact that the other nerves to be described below appear to be distributed only to the vas deferens and the epididymis lends support to Hovelacque's idea that the nerve supplies to the testicle and the epididymis are distinct and separate. Another finding which supports this view will be mentioned later.

(2) *The intermediate or middle spermatic group.* In the majority of specimens a nerve was discovered arising by a varying number of rootlets from the side of the superior hypogastric plexus, or from the upper end of the hypogastric nerve of the same side, and this nerve was invariably associated with a twig passing to the ureter. The two commonest arrangements are shown in Plate IV, fig. 4, and Plate VII, fig. 9. In Plate VII, fig. 9, the nerve to the ureter and the middle spermatic nerve are seen arising from the lower end of the superior hypogastric plexus, and uniting a short distance from their origin by a short cross branch. The spermatic nerve eventually divided into three main branches, two entering the epididymis directly and the third passing to the vas deferens. In Plate IV, fig. 4 there are two middle spermatic nerves, the upper arising from the superior hypogastric plexus and the lower from the hypogastric nerve. The upper arose by multiple rootlets which soon united to form a single nerve. A twig passed to the ureter from the same region of the hypogastric plexus, and lower down a second ureteral branch was given off from the terminal part of the superior hypogastric plexus. The second middle spermatic nerve in this specimen arose from the hypogastric nerve, in conjunction with another nerve (which passed antero-internal to the ureter) destined for the supply of the ureter, seminal vesicles and vas deferens; one of the larger branches accompanied the vas deferens and united near the internal abdominal ring with the second spermatic nerve mentioned above. Thereafter the common nerve so formed could be traced downwards as far as the epididymis. Another less common arrangement, which was described when discussing the innervation of the ureter, is shown in Plate VI, fig. 8. An interesting condition is shown in text-fig. 11, where the nerve under discussion arose from the hypogastric nerve and accompanied an abnormal spermatic artery, which originated from the hypogastric artery. The spermatic vein was normal in position and was accompanied by two spermatic nerves—one from the renal plexus and another connected with the intermesenteric nerves.

The middle spermatic nerve, with the exception of the specimen shown in Plate IV, fig. 4, has always been single, and passes downwards and outwards

to the internal abdominal ring where it becomes associated with the other structures which constitute the spermatic cord. It finally splits up into several branches which are distributed to the epididymis and the beginning of the vas deferens. It communicates with the nerve passing along the vas from the inferior hypogastric plexus, but no macroscopic communication could be discovered between these nerves and the nerves which were described above as the superior spermatic nerves. The testis and epididymis of the specimen shown in Plate VII, fig. 9 were cut in serial sections and examined microscopically. Branches of the nerve just described could be traced in the epididymis only, and none could be followed into the actual testicle. Too much stress cannot be laid on a single observation, but nevertheless the localisation of this nerve to the epididymis was significant, especially when considered along with the macroscopic findings in the other cases.

(3) *The inferior spermatic group.* The inferior spermatic nerves arise from the nerve loop which often surrounds the lower end of the ureter, or spring directly from the inferior hypogastric plexus (Plate VII, fig. 9, and Plate VIII, fig. 10). A leash of branches (Plate VI, fig. 8, text-fig. 9) passes to the vas deferens from the peri-ureteral nerve loop and from the adjacent portion of the inferior hypogastric plexus. Most of these are fine in size and are distributed to the vas in a fan-shaped manner, but one member of the group is usually distinctly larger and has a more extensive distribution. It arises from the anterior part of the loop, or from the antero-superior part of the inferior hypogastric plexus, by a single root or by several rootlets which coalesce to form one or rarely two nerves. The nerve so constituted accompanies the vas closely and supplies filaments to it, but it may be separated off readily in the course of a dissection, and it can be traced with ease as far as the epididymis which it supplies. It communicates with the middle spermatic nerve, but apparently never communicates with the superior spermatic nerves. Variations of this arrangement are seen in Plate IV, fig. 4, and Plate VI, fig. 8 and they have already been described.

SUMMARY

1. Many of the failures following operations on the autonomic nervous system have been due to inaccurate anatomical knowledge.

2. A description is given of the autonomic nerves and plexuses from which the nerves to the kidney, ureter, testicle and epididymis are derived.

3. The kidney receives a nerve supply from:

(*a*) The aortico-renal ganglion.
(*b*) The middle and inferior splanchnic nerves.
(*c*) The intermesenteric nerves.
(*d*) The lumbar sympathetic chain.

4. Stripping of the renal artery will not secure complete renal denervation in every case.

5. The ureter receives nerve filaments from:

(*a*) The lower fibres of the renal plexus or the intermesenteric nerves.

(*b*) The superior hypogastric plexus or the upper end of the hypogastric nerve.

(*c*) The lower end of the hypogastric nerve and the upper part of the inferior hypogastric plexus.

6. The inferior ureteric nerves are closely associated with filaments which supply the vas deferens and seminal vesicle.

7. The testicle and epididymis receive nerves from:

(*a*) The intermesenteric nerves and renal plexus.

(*b*) The superior hypogastric plexus or the upper end of the hypogastric nerve.

(*c*) The inferior hypogastric plexus.

8. Testicular afferents apparently enter the cord at a higher level than is commonly stated.

9. In one dissection a direct communication was discovered between the superior testicular nerve and a nerve supplying the third part of the duodenum. Such a linkage may explain the sickness associated with testicular injuries.

10. It is probable that the nerve supplies to the testicle and epididymis are distinct and separate.

This investigation was carried out in the Department of Surgery, Aberdeen University, and the expenses were partly defrayed with the aid of a grant from the Medical Research Council. I am deeply indebted to Prof. J. R. Learmonth for his invaluable help and encouragement.

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EXPLANATION OF PLATES AND TEXT-FIGURES

1, Semilunar ganglion.
2, Aortico-renal ganglion.
3, Renal ganglion.
4, Phrenic ganglion.
5, Intermesenteric nerves.
6, Inferior mesenteric plexus.
7, Superior hypogastric plexus.
8, Hypogastric nerves.
8 *A*, Middle hypogastric plexus.
9, Inferior hypogastric plexus.
10, Superior splanchnic nerve.
11, Middle splanchnic nerve.
12, Inferior splanchnic nerve.
13, Lumbar sympathetic trunk.
14, Rami passing to intermesenteric nerves.
15, Rami passing to superior hypogastric plexus.
16, Nerves from aortico-renal ganglion to renal plexus.
17, Branches from intermesenteric nerves to renal plexus.
18, Nerves connecting renal plexus with intermesenteric nerves and inferior mesenteric plexus.
18 *A*, Branch to renal plexus from lumbar sympathetic chain.
19, Nerve loop connecting coeliac and superior mesenteric plexuses to inferior mesenteric plexus.
20, Colonic nerves accompanying middle colic artery and its branches.
21, Colonic nerve accompanying ascending branch of left colic artery.
22, Colonic nerves accompanying inferior mesenteric artery and its branches.
23, Nerves connecting superior hypogastric plexus to inferior mesenteric plexus.
24, Colonic branches from hypogastric nerve and inferior hypogastric plexus.
25, The special nerve arrangement described by Stopford and Telford. The short branch indicated by an asterisk in text-fig. 4 arose from the left hypogastric nerve, but in the photograph (Plate I, fig. 1) the point of origin is concealed by a piece of black paper.
26, The nerve described by the writer (see *Edin. med. J.* 1935, XLII, 11). In (Plate I, fig. 1) it is overlapped for a small part of its course by a piece of black paper.
27, A nerve formed by the junction of two nerves similar to the above (26).
28, Loop connecting one of the intermesenteric nerves to the inferior mesenteric plexus.
29, Superior spermatic nerves.
30, Middle spermatic nerve(s).
31, Inferior spermatic nerve.
32, Branch of superior spermatic nerve described on page 26 of the text.
33, Ganglionic enlargements on superior spermatic nerve.
34, Superior ureteral nerves.
35, Middle ureteral nerves.
36, Inferior ureteral nerves.
37, Nerve loop in front of the lower end of the ureter.
38, Nerves to vas deferens.
39, Pelvic splanchnic nerve(s).
A, Appendix.
B, Bladder (split in median sagittal plane).
C, Caecum.
D, Duodenum (third part turned upwards).
E, Epididymis.
I, Ileum (cut across near caecum).
K, Kidney.
P, Pancreas.
R, Rectum.
S, Suprarenal.
T, Testicle.
U, Ureter.
V, Vas deferens.
AC, Ascending colon.
TC, Transverse colon.
DC, Descending colon.
SC, Sigmoid colon.
IM, Inferior mesenteric artery.

TC
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Fig. 1.

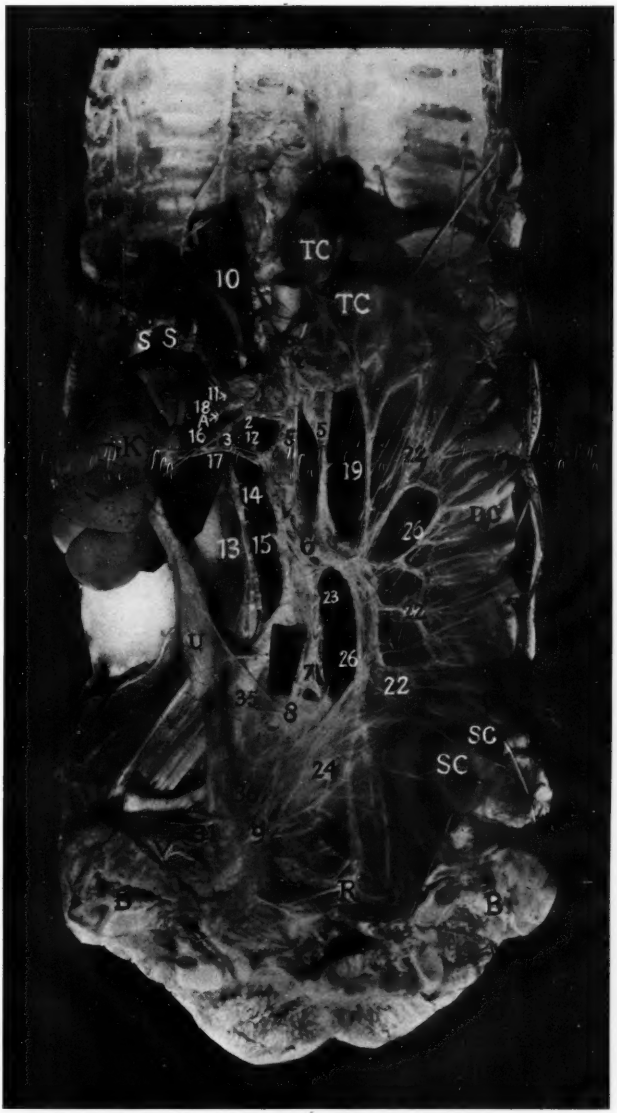

Fig. 2.

Fig. 3.

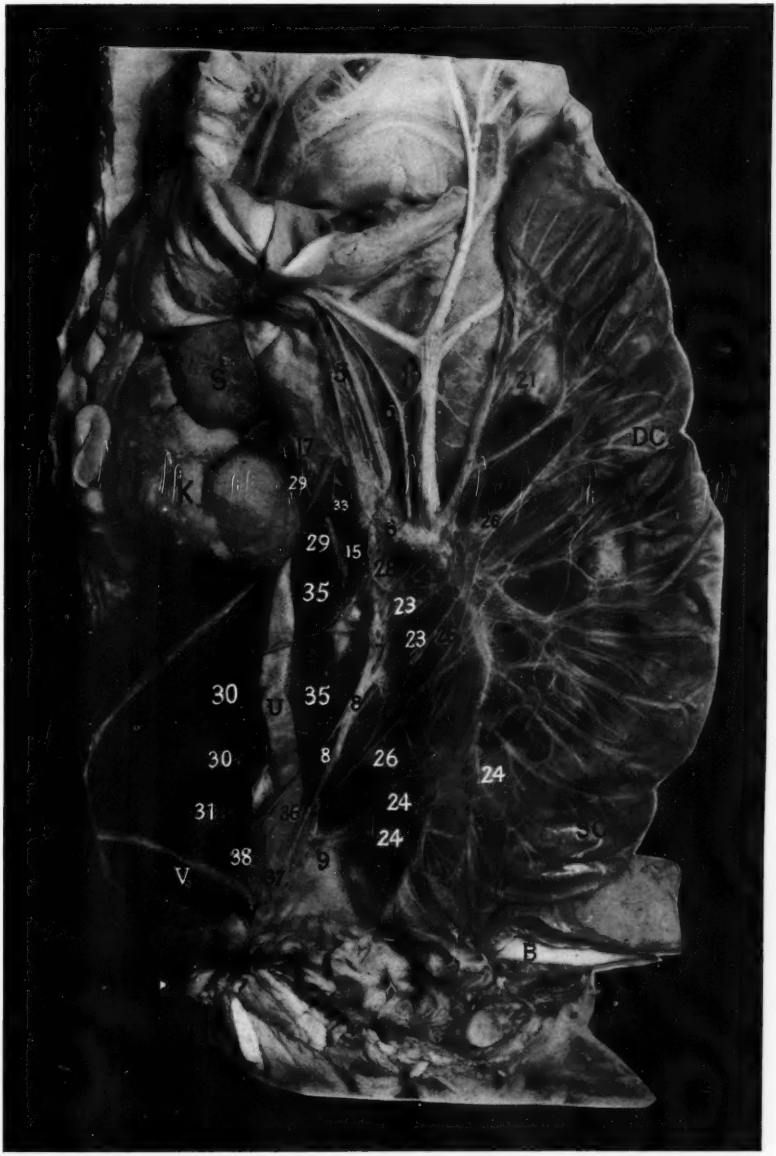

Fig. 4.

Fig. 5. Fig. 6.

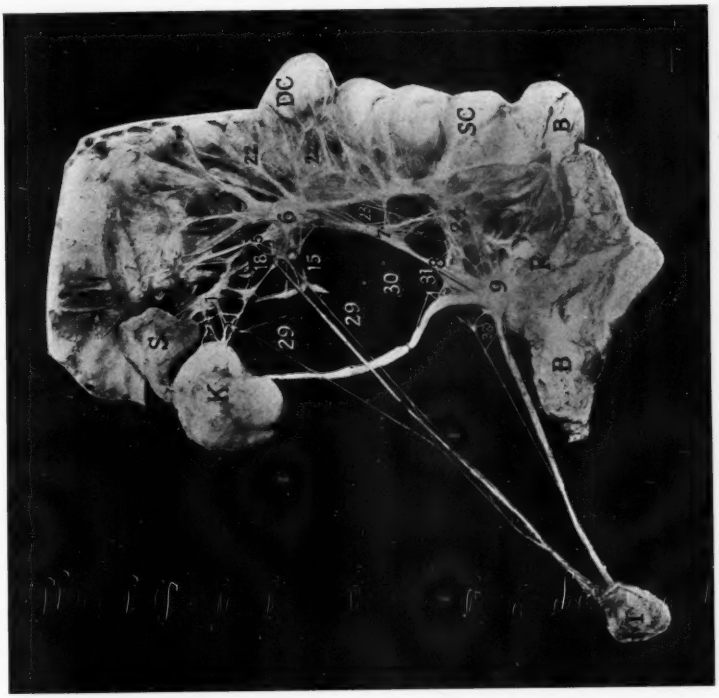

Fig. 8.

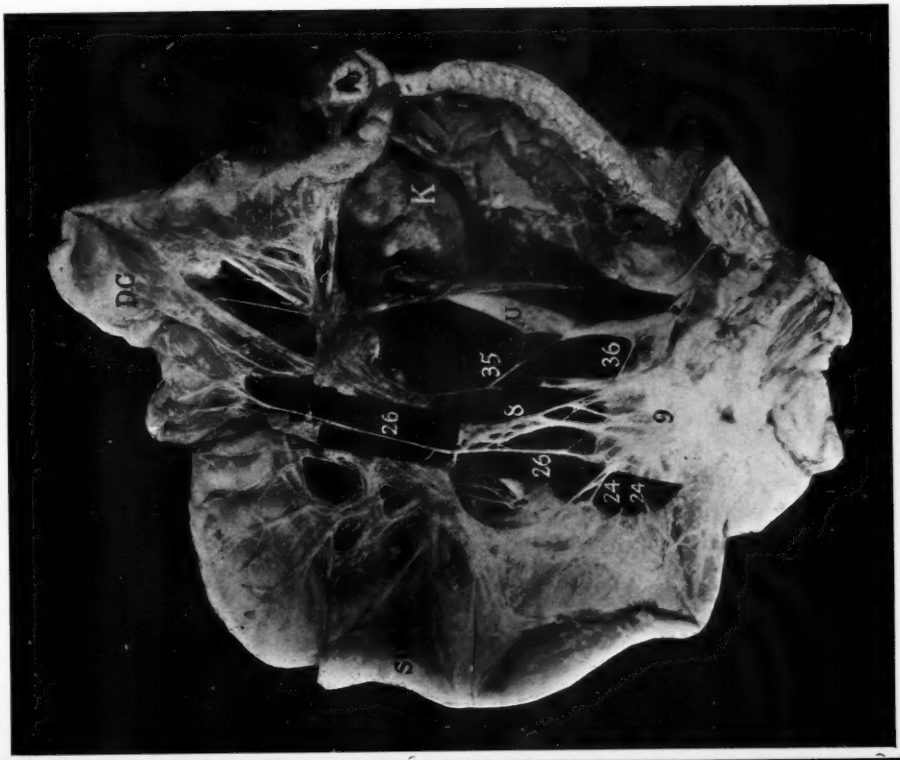

Fig. 7.

Fig. 9.

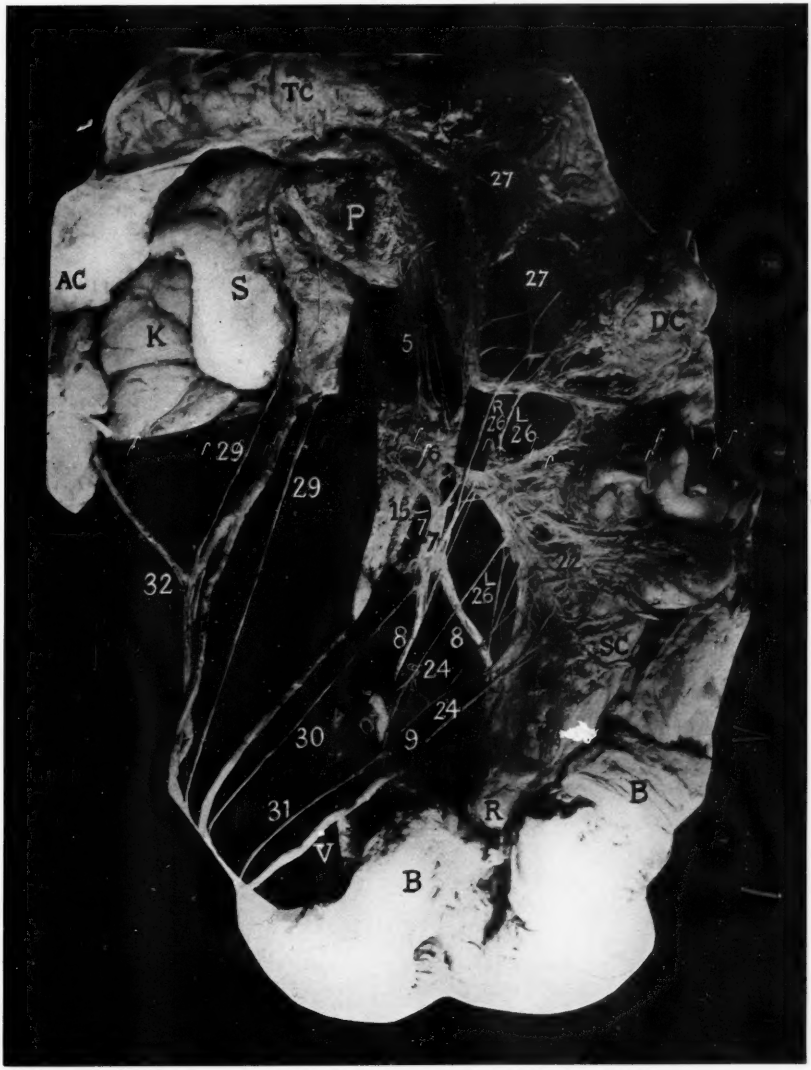

Fig. 10. The letters R and L above the nerves marked 26 indicate the right and left nerves respectively.

A CONSIDERATION OF THE CONSTANCY OF MUSCULAR NERVE SUPPLY

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INTRODUCTION

It has been known for over half a century that muscles tend to be conservative in their nerve supplies. If in one animal a particular muscle, say the praesemimembranosus, is supplied by a nerve from the sacral plexus, it is usually found that its homologue in a second animal is supplied from the same source, and not by a branch, say, of the obturator nerve. Yet such exceptional innervations seem to occur, sometimes in animals belonging to the same order, and their significance has aroused the greatest interest among comparative myologists. For upon the significance and frequency of these exceptions depends the whole question of the reliability of an appeal to the nerve supply when the homologies of the muscles are doubtful. If the nerve supplies of muscles never change, or if such changes are very infrequent, then the nerve supply is a reliable guide to muscle homology. But if, with specialisation or migration of the muscles, their nerve supplies are likely to change, then in just those cases of doubtful homology where the nerve supply would be particularly useful as a criterion, this criterion may be most likely to lead to error.

The hypothesis that there is a very strict association between a muscle and its nerve supply was elaborated in great detail by Fürbringer, and has become generally known as the Fürbringer hypothesis. Since this forms the basis of nearly all later work on the nerve supplies of muscles, it is necessary to state it clearly, particularly as it has seldom been discussed as a whole. The development of his theories occupies many sections of Fürbringer's great works, on the anatomy of birds (1888), and on the spino-occipital region of Selachians (1897), but no summary of the whole system of interconnected theories has been published either by Fürbringer or by any other author. In this paper, then, an account of the hypothesis will first be given, and this will be followed by an account of the criticisms that have been advanced, particularly by comparative myologists. To clarify the points at issue a new series of dissections will be described and figured, mostly illustrating classical cases of change of nerve supply. Finally, the more recent neurological work will be discussed in its relation to the nerve supplies of muscles.

THE FÜRBRINGER HYPOTHESIS

This account of the Fürbringer hypothesis will be taken, where possible, from the later paper (1897), but, where the earlier work (1888) is more detailed, reference will be made to it. The two papers are complementary, for Fürbringer in the later paper stated clearly that he had found not the least reason to alter his opinions in the time which had elapsed between them.

The central proposition was that of the unbroken protoplasmic continuity of muscle and nerve elements throughout both phylogenetic evolution and individual development. This "Neuromuskeltheorie" was originally developed by Kleinenberg in his studies of the neuromuscular system of *Hydra*, and was later extended by Gegenbaur to include other kinds of animals. The cells of the dividing ovum were said not to be cut off completely from one another during division, but to remain connected by protoplasmic bridges, which in the case of those cells which gave rise to the muscle fibres and to the ganglion cells persisted into adult life (1888, p. 897).

Similarly in Vertebrates Fürbringer supposed that the primordia of the nerves were at first fine protoplasmic bridges between the embryonic cells which were to give rise to the ganglion cells and to the muscle fibres, and that these bridges had persisted from the earliest divisions of the ovum. During development these connections might become stretched, but were never broken (1897, p. 744). The bridges were at first too fine to be demonstrable by any of the staining methods in use (methods of His, Golgi, Erlich) (1897, p. 736). Thus the accounts given by His and Cajal of a free outgrowth of nerve fibres from the spinal cord, and their secondary connection with the muscles, were erroneous, for in fact there was a primary connection which became visible in stained preparations only when it became fully differentiated into nervous tissue late in development. Further the cone of growth, which Cajal and others interpreted as the actual growing point of a nerve fibre as it made its way through the tissues towards the muscle it was to supply, was capable of an alternative interpretation. Fürbringer supposed that it was the result of a wave of differentiation which was moving peripherally along a preformed protoplasmic bridge, leaving behind it a thickened fibre now recognisable as a nerve, where before there had been only an invisible primordium (1897, p. 737). Even in the adult, where the motor end plate was fully differentiated, the nerve was connected to the muscle by protoplasmic bridges, but these were too fine and too short to be visible by direct microscopical observation (1897, p. 743).

It is evident then that each muscle had at all stages in its development a fixed and unchangeable connection with its nerve supply. However much the fibre might wander in the course of its development, it took its nerve supply with it, and this could be used as an infallible guide in tracing the embryonic origin of a fibre. In the limbs it was usual to find fibres, supplied by nerves of several different segments, grouped together to form a single muscle. In

this event the nerve supply could be used to determine with certainty the segmental origin of the embryonic tissue which gave rise to the muscle, even when, as was usually the case, this could not be determined by direct observation. Hence the nerve supply of a muscle was the most important and reliable criterion in the determination of its homologies (1888, p. 942).

For two muscles to be considered homologous, not only had their structure and relations to be similar, but their nerve supplies had also to be similar, that is, to have the same segmental origin and to arise from the same plexus. But in fact, on comparing one animal with another, using even closely related species, though the muscles were usually found in very similar positions and carrying out the same mechanical functions, it was often found that their nerve supplies were not precisely similar, so that although apart from a study of their nerve supplies they might have been considered homologous, there could in fact be only a parhomology between them. The most striking instances were those in which a single muscle was supplied by nerves from two different plexuses as, for instance, the trapezius, which was usually supplied both by cerebral and by cervical nerves, but in very varying degree, so that sometimes the cervical nerves were entirely absent (Amphibians), and sometimes the cerebral part was much reduced (Birds). Again in Monotremes the tibialis anterior was supplied by the femoral nerve, in placental Mammals by the peroneal nerve. Thus in most animals a set of muscles was developed, each of which resembled more or less closely a similar muscle in related animals, but the individual muscles were often built up from dissimilar embryonic components. This process of development of similar muscles from dissimilar material was called by Fürbringer a vicarious development of muscles, and appeared to be very common (1888, pp. 967 ff.).

CRITICISMS OF THE FÜRBRINGER HYPOTHESIS

It will be understood that the Fürbringer hypothesis was a very complete and consistent series of related theories, and was a logical development of the earlier Neuromuskeltheorie of Kleinenberg and Gegenbaur. But by the time that Fürbringer's work was completed, an alternative hypothesis of the free outgrowth of nerves from the spinal cord and their secondary union with the muscle fibres was very well supported, and was discussed by Fürbringer in great detail. His (Fürbringer, 1897, p. 733) had observed the nerves in the human embryo ending freely in the tissues before they had reached the muscles, and had described them as being from the beginning of measurable size. He had, on the other hand, not found any trace of a protoplasmic bridge or other preformed pathway leading from the spinal cord to the muscles, and he had suggested that the nerves were guided through the tissues by resistances such as the bones and blood vessels. Cajal (Fürbringer, 1897, p. 734) had described the cone of growth at the actual free end of a growing nerve before it had reached the muscle fibre, guided perhaps by a chemiotactic response to a substance synthesised in the muscle fibre. It has been seen that Fürbringer

advanced criticisms of their observations, based on the supposed inadequacy of microscopic technique. Later work, however, particularly that of Harrison (1910), who showed experimentally that nerves will grow out freely from an isolated piece of spinal cord, has supported the view advanced by His, that there is a secondary union between the nerves and the muscle fibres. This is now generally accepted, though Graham Kerr (1919) in his work on the embryology of *Lepidosiren* has again supported the older view that there is a primary connection.

Again the criterion of nerve supply has often been found difficult in its application to comparative myology, particularly that of the Reptiles. Gadow (1882, p. 456), for example, stated clearly that since he had found instances where one and the same muscle was supplied from different nerve plexuses in different species of Reptiles, the innervation could not be used as the chief guide in the establishment of muscle homologies. After the publication of the Fürbringer hypothesis Cunningham (1890), in a very important paper, re-examined the evidence. He found that though, as a rule, homologous muscles in different animals were supplied by the same nerve trunks, there were several striking exceptions. From his own work on the muscles of the feet of Mammals, he quoted instances where either the medial or the lateral plantar nerve appeared to have overstepped its usual limits, so as to "lay hold upon" some muscle which it did not usually supply. Thus in the elephant, hyrax and beaver the medial plantar had invaded the muscular territory usually supplied by the lateral plantar, while in the fox-bat the lateral plantar had invaded that of the medial plantar nerve. Other examples discussed by Cunningham will be mentioned later, when the new dissections are described.

Now Fürbringer, as has appeared, had decided that these changes in the nerve supply of apparently homologous muscles were really the results of the vicarious development of muscles, that is, of the process whereby the muscles of two animals may resemble one another very closely in form and position, but may be built up from quite different tissue in the embryo, so that they are not truly homologous with one another. Cunningham, on the other hand, assumed that the muscles themselves were homologous structures, but that the number of muscles supplied by any particular nerve might vary. Unfortunately, although Cunningham gave a good account of Fürbringer's views on the question of the primary or secondary connection of nerve fibres and muscle fibres, he did not describe in the same detail Fürbringer's theory of the vicarious development of muscles. As a result of this, it is possible that a false impression of the Fürbringer hypothesis has grown up. It is often supposed that Fürbringer, in stating that a muscle had a fixed and unalterable relation to its nerve supply, meant that any particular muscle, say the deltoid, must always be supplied exclusively by a particular nerve, in this instance the axillary, and could never be supplied by any other nerve, say an anterior thoracic nerve. In fact Fürbringer gave a very complete list of the variations in the nerve supplies of muscles known when he wrote, without questioning their reality. But he

did not consider that two deltoids which had different nerve supplies were fully homologous structures, but only parhomologous muscles, whose similarity was the result of a vicarious development.

Cunningham, then, decided that muscles were not always supplied by the same nerve trunks, but he left it an open question whether the nerve supply of any particular muscle fibre always arose from the same ganglion cell. He suggested that possibly the anomalous nerve supplies which undoubtedly occur were due to a process of nerve transference, whereby a nerve might follow an unusual course without any change in the position of the ganglion cell in which it originated or of the muscle fibre which it supplied. It was possible, he thought, that this transference might take place within the spinal cord itself, so that the nerve, although it arose from a ganglion cell in the old position, might emerge by a root lying above or below the one in which it was usually found.

The theory of nerve transference from one nerve trunk to another was also accepted by Eisler (1895), in his work on the lumbosacral nerves. He found that the plexuses which gave rise to the trunks were connected with one another through the nervus furcalis, so that a transference of fibres by this route would be possible without any visible disturbance of the plexuses. But Frets (1909, p. 89) found a specimen of *Ornithorhynchus* in which, as compared with other animals, the obturator nerve had displaced the sacral nerves in the supply of certain muscles, although there were no connections between the obturator and sacral plexuses, so that there could have been no nerve transferences. Frets associated himself with Fürbringer in concluding that the muscles supplied by different nerves were not homologous and had different origins in the embryo.

Bardeen (1907, pp. 268, 372) emphasised the great variability in the arrangement of the nerves supplying the limb muscles in human embryos. Not only the pattern of the plexuses and the paths of the motor nerves through the nerve trunks, but also the segmental origin of the nerves to any particular muscle varied from individual to individual. He thought it probable that the nerves extended into the musculature in response to some specific attraction, and that the supply of particular muscles by particular nerves was due to the fact that they lay in the path of the nerves as these grew out into the limbs.

Ribbing (1908), working on Amphibia and Reptiles, found that the extensors of the forearm were sometimes supplied by the deep radial nerve alone, sometimes by the superfieial radial as well, and sometimes by nerves from the flexor region which reached them by passing between the radius and ulna. Similarly in the leg the extensors might be supplied entirely by the peroneal nerve, or by the saphenous nerve as well, or by a nerve passing from the flexor region between the tibia and fibula. Since the muscles were fairly constant in arrangement it seemed to him that the innervations were more variable than had previously been believed.

Edgeworth (1911) collected fifteen examples of cranial muscles which had

changed their nerve supply, emphasising the value of embryological evidence in confirming the accuracy of his interpretations. He found that he could explain fourteen of these on the supposition that, when a muscle spreads into one or more neighbouring segments, it tends to be innervated by the corresponding nerve or nerves. Later (Adams, Wheeler and Edgeworth, 1929) he showed that the nerve trunks themselves are variable in their relations to surrounding structures. In the study of the mandibulo-auricularis he found that too great reliance had been placed by Huber on the arrangement of the nerve supply, and that this had led to a misunderstanding of its development, which became quite clear when the embryology was studied directly. Rabl (1916) again, working on Reptiles, decided that the course taken by a motor nerve to a muscle is inconstant, but supported the view suggested by Cunningham, that though nerve transference is common, there is a close association in phylogeny between particular muscles and particular groups of ganglion cells.

Yet in spite of the criticisms advanced by Cunningham, Ribbing, Edgeworth and others, comparative myologists have on the whole tended to rely on nerve supplies in the determination of muscular homologies, particularly those of the groups of muscles supplied by the larger nerve trunks. Gregory and Camp (1918), in their review of the myology of the limbs, classified the muscles by their nerve supplies, a procedure which would clearly be meaningless if the nerve supplies were expected to vary to any significant extent from group to group. Romer (1922) and Appleton (1928) have followed them, though both authors have quoted examples of variation in nerve supplies. Further, by stimulation of the nerve roots within the spinal canal, it has been possible to demonstrate that the platysma of the neck is always supplied exclusively by the facial nerve, and never, as had been claimed by some authors, by the cervical nerve (Huber and Hughson, 1926; Smith, 1931). It may be that some other difficult questions of nerve supply will be cleared up by this technique, though it seems unlikely that much new knowledge will be gained in the case of large muscles whose nerve supply can readily be determined by gross dissection.

Weed (1927) has summed up the present position in the statement that "though the concept of Fürbringer has been repeatedly attacked, it has withstood all criticism and to-day is fairly generally accepted."

Recently Addens (1933), working chiefly on the cranial nerves, has shown that Cunningham's conception of nerve transference within the central nervous system can be extended to include a large number of anomalous nerve supplies. In the cockatoo, for instance, part of the motor seventh nerve, which arises from a nucleus in the usual position, emerges with the fifth. From his own work and from that of other authors, Addens has collected together many examples of this kind of nerve migration, which he calls central anastomosis, and has suggested that they may be explained on the principle of fasciculation, whereby nerve fibres which conduct impulses simultaneously in the same direction, or possibly in opposite directions, tend to become grouped together in bundles.

Central anastomosis may prove to be of some importance in the arrangement of the nerves which supply the limbs, and will certainly tend to weaken any attack which may be made on the Fürbringer hypothesis. Addens himself, however, though he believes real change of innervation to be rare, quotes two examples where it seems to have occurred: the electric organ of the star-gazers, and the tail muscles of Mammals.

However, from the point of view of the myologist interested primarily in the musculature of the limbs, it may be many years before sufficient information is collected to allow a judgment to be made as to whether there is a close relation between particular groups of ganglion cells in the spinal cord and particular muscles in the limbs. It has been seen that, on the whole, reliance has been placed on the nerve supply in the determination of homologies, on the assumption that the arrangement of the nerve trunks is constant. In the following pages an attempt will be made to evaluate this doctrine in some classical cases, and to weigh it against the alternative theory that the muscles are supplied by the nerve trunks near whose path they happen to lie during development.

DISCUSSION OF SPECIAL EXAMPLES

The most famous of the cases of aberrant nerve supply is that of the tibialis anterior and extensor hallucis longus in *Ornithorhynchus*, brought forward by Ruge (1878) at a time when nerve supplies were already accepted as reliable guides to the homologies of muscles, but before Fürbringer had elaborated his detailed hypothesis. Fig. 1 shows a dissection of the region under discussion, with the extensor muscles and the cutaneous and muscular nerves. From the medial to the lateral side are found the tibialis anterior, extensor hallucis longus, extensor digitorum longus, extensor digitorum communis brevis, peroneus brevis and peroneus longus lying in the typical mammalian order as described by Ruge. The peroneus brevis was called by Ruge the extensor brevis digiti V, and was considered by him as being only partially comparable to the peroneus brevis of other Mammals. The other names used are those given by Ruge, the extensor digitorum communis brevis being so called although it arises from the bones of the leg and not from the bones of the tarsus.

The arrangement of the muscles is fairly typical of the Mammalia as a whole, apart from minor specialisations such as the expansions given off by the extensor digitorum brevis to the medial and lateral borders of the foot, apparently homologous with the ligaments which hold the extensor tendons in place in other Mammals. Apart from their nerve supplies there would be no difficulty in supposing the muscles strictly homologous. But in typical Mammals all the muscles are supplied by the peroneal nerve, while in *Ornithorhynchus* the tibialis anterior and the medial part of the extensor hallucis longus are supplied by a branch of the femoral nerve which passes over the vasti and the lateral femoral condyle and deep to the ligament which connects the patella and fibula, and which gives partial attachment to the insertion of

the biceps and the origin of the tibialis anterior. Reaching the space between the tibialis anterior and the extensor hallucis longus it divides into several branches for their supply. The peroneal nerve, after giving off its superficial branch, passes through the peroneus longus, and then between it and the peroneus brevis to the extensor digitorum longus which it pierces, and ends in

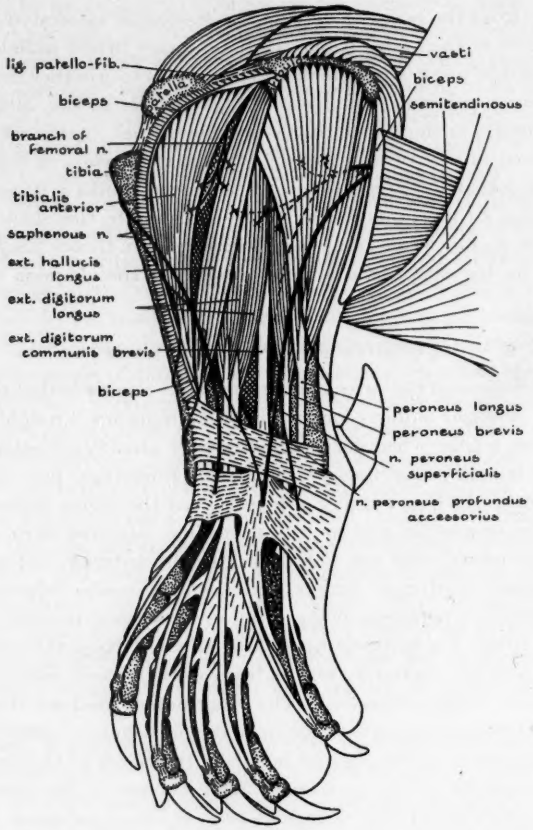

Fig. 1.

the extensor hallucis longus, having supplied all the muscles lying lateral to it. No deep peroneal nerve is given off, but an accessory deep peroneal nerve, comparable to that found in most Mammals but not in Man, reaches the foot between the extensor digitorum communis brevis and the peroneus brevis.

Ruge, having described these conditions, decided at once that since the tibialis anterior of *Ornithorhynchus* was not supplied by the peroneal nerve, it could not be homologous with the tibialis anterior of other Mammals.

Similarly, there was an incomplete homology between the extensor hallucis longus of most Mammals and that of *Ornithorhynchus*, which was only partially supplied by the peroneal nerve. He hoped that an explanation of the curious conditions in *Ornithorhynchus* would be forthcoming when new studies had been made on the Reptiles. In the meantime he suggested that, in the ancestors of the Mammals, the extensors of the thigh stretched distally over the leg and were supplied by the femoral nerve, but that in the modern Mammals they had shrunk back on to the thigh, and their place had been taken by flexor musculature which had migrated medially from the lateral side of the leg carrying its nerve supply, the peroneal nerve, with it. Further he suggested that the saphenous branch of the femoral nerve, which in *Ornithorhynchus* was very large and supplied the medial side of the foot, giving branches to the first two interdigital clefts, in other Mammals became reduced to its typical mammalian condition. Fürbringer (1888), following Ruge, agreed that the muscles in Monotremes and in other Mammals were not homologous, and explained their innervations as being the result of a vicarious development.

Cunningham (1890), on the other hand, found the arrangements of the muscles in *Ornithorhynchus* and in other Mammals so similar that he accepted them as homologous, in spite of their differences in nerve supply. He thought that Ruge had been right in supposing that the extension of the femoral nerve into the leg in Monotremes was primitive, but differed from Ruge in his suggestion that the muscles had remained in place while the peroneal nerve usurped the place of the femoral in other Mammals, probably by a process of nerve transference. Eisler (1895) and Ribbing (1908) also believed that the muscles had remained in place, but Frets (1909) returned to the older view of Ruge and Fürbringer that the muscles were not homologous structures, but were built up from dissimilar tissue in Monotremes and in other Mammals.

Other authors have been interested in these muscles, but have been unable to find any explanation of their peculiarities. Appleton (1928) gave this and Cunningham's example from the foot as instances of a change of nerve supply, rare in any case, and remarked that their significance could not yet be estimated. But it may be useful to compare the Monotremes with the more primitive Reptiles.

Fig. 2 shows a dissection of the leg and foot of an iguana. The muscles on the extensor aspect are, following the terminology used by Rabl (1916), a tibialis anterior, extensor digitorum communis, peroneus brevis and peroneus longus. The tibialis anterior passes from the tibia to the first metatarsal bone, the extensor digitorum communis from the lateral condyle of the femur to the third metatarsal, the peroneus brevis from the fibula to the fifth metatarsal, and the peroneus longus from the lower end of the femur and the head of the fibula to the tarsal bones. The fibular collateral ligament of the knee joint appears to be not yet differentiated from the origin of the peroneus longus, its place being taken functionally by a femorofibular ligament which lies in the position of, and is apparently comparable to, the tendon of origin of the

popliteus in Mammals. Passing from the lower end of the fibula to the dorsum of the foot are the various slips of the fibulo-tarso-digitalis dorsalis, and on the foot itself are the short extensors of the toes, the interossei and the short muscles of the lateral side of the foot.

The same general arrangement is found also in *Sphenodon*, in the crocodile and in other lizards, as well as in the Amphibians (Gadow, 1882; Ribbing,

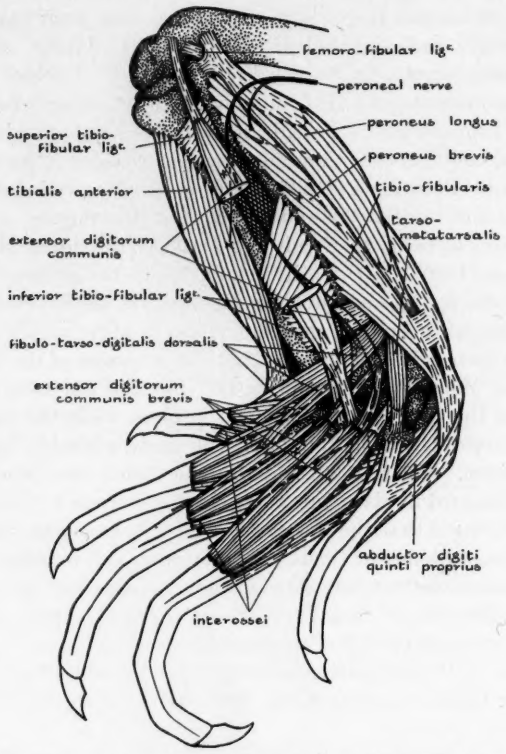

Fig. 2. Extensor muscles of the leg and foot of *Iguana tuberculata*.

1908), so that it appears to be the primitive tetrapod state. Whether it is primitive and representative of the state in the reptilian ancestors of the Mammals, or whether the unbroken muscles passing from the leg to the phalanges in the Mammals are the more primitive, and have subsequently become divided in the modern Reptiles, cannot yet be decided, though the first alternative appears the more probable. With the suppression of two phalanges from the fourth toe and one from each of the third and fifth in the evolution of the Mammals, the musculature may have undergone considerable changes, and it is probable that the long muscles of Mammals are neotenic in

their structure, rather than truly primitive. However this may be, the condition of the nerve supply is unequivocal: all the extensors are supplied by the peroneal nerve, none by the femoral. The femoral innervation of the tibialis anterior and extensor hallucis longus of Monotremes is therefore probably secondary. Ribbing (1908) found femoral innervations of the extensors in *Salamandra* and in a few specialised lizards, and from analogies with the innervations of the muscles of the forearm supposed them to be primitive, but it would seem more probable that they are due to parallel evolution in divergent groups.

Now it has been seen that the reason for a change of nerve supply suggested by Edgeworth (1911) and others, was the migration of the muscle in question towards the trunk from which it took over its supply. But in *Ornithorhynchus* the muscles have not migrated to any extent, so this suggestion cannot be directly applied. On the other hand the head of the fibula has undergone a peculiar expansion, which has carried the neck far away from the shaft of the tibia. The peroneal nerve winds round the outer side of the neck, and has been carried out with it, so that the most medially lying muscles have become widely separated from their usual nerve supply, and have come to lie relatively, though not absolutely, nearer the femoral nerve. Thus they can acquire their nerve supply by a new path from the femoral, and this change can be brought into line with others that have been explained by an alteration in the relative position of muscle and nerve. The same argument would apply to the muscles of *Echidna*, where the femoral nerve supplies the tibialis anterior, the extensor hallucis longus and part of the extensor digitorum communis (Ribbing, 1908).

THE LONG MUSCLES OF THE THIGH

Interest in the pelvic muscles of Reptiles and Mammals has been aroused by the attempted reconstruction of the muscles of extinct animals by a study of the markings on their bones made by Gregory and Camp (1918) and their followers. The pelvic muscles form an excellent field for the study of nerve supplies, as the femoral, obturator and sciatic groups of nerves are always very distinct, and as the pelvis has not undergone such radical changes in structure as has the shoulder girdle in passing from the reptilian to the mammalian type. The terminology will follow that of Romer (1922) as far as possible. For the exceptions, and for a detailed discussion of the homologies of the muscles, see Haines (1934). The pelvic muscles of Amphibians are not discussed, as they are extremely difficult to compare with those of other Tetrapods. Probably, even in the case of the Caducibranchiata, they are neotenic in structure.

Figs. 3–6 show the pelves and the attached long thigh muscles of an iguana, a crocodile, an *Ornithorhynchus* and a squirrel (*Sciurus*). In *Iguana* the femoral nerve gives four muscular branches, one each to the femorotibialis and ambiens which it supplies completely, and one each to the iliotibialis and iliofemoralis which also receive branches from the sacral nerves. The obturator nerve supplies

the flexor intercapitalis anterior completely, and partially supplies the adductor femoris and puboischiotibialis which also receive branches from the sacral nerves. The sacral nerves emerge partly dorsal and partly ventral to the caudofemoralis and caudoiliofemoralis. The dorsal group includes the partial supplies of the iliotibialis and iliofemoralis, and the entire supply of the iliofibularis; the ventral group, the n. ischiadicus ventralis, includes the partial supplies of the puboischiotibialis and the adductor femoris, and the entire supplies of the flexor tibialis externus, flexor tibialis inferior, flexor tibialis genicularis and flexor intercapitalis posterior.

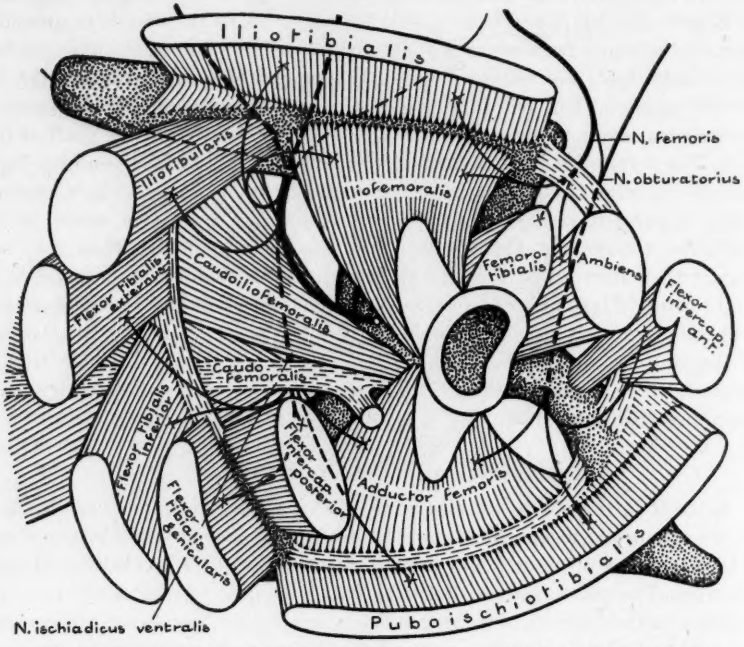

Fig. 3. Long thigh muscles of *Iguana tuberculata*, cut off short, in lateral view.

Three muscles in the crocodile have changed their nerve supplies completely: the adductor tibialis, the flexor intercapitalis anterior and the flexor tibialis genicularis. The adductor tibialis is not represented in the iguanas figured here and by Romer (1922), but was present in a specimen dissected by Haines (1934), and is commonly present in lizards and in *Sphenodon*. In these animals it is inserted on the tibia as a flexor, and is supplied by the obturator nerve; in the crocodile it is inserted as an extensor and is supplied by the femoral. In the crocodile this muscle has been considered as a part of the ambiens on account of its nerve supply, but it seems more probable that it is the homologue of the common reptilian adductor tibialis which has changed its

nerve supply as the muscle has migrated from the flexor to the extensor region.

The flexor intercapitalis anterior is somewhat doubtfully identified in the crocodile, as it has undergone extensive migrations from the pubis to the posterior border of the ischium. In the lizard it is supplied by the obturator nerve, in the crocodile by the nervus ischiadicus ventralis, apparently as a result of its migration from one nervous field into another.

The flexor tibialis genicularis can be identified with more certainty by its characteristic attachment and relations, which are very similar in the lizard

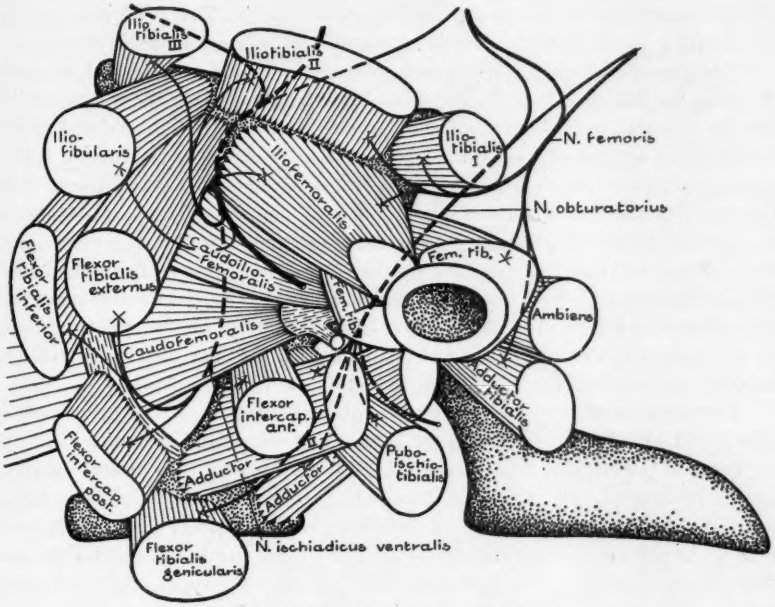

Fig. 4. Long thigh muscles of *Crocodilus niloticus*.

and crocodile. The nerve supply has changed from the n. ischiadicus ventralis to the obturator. Here there has been no migration of the muscle, but if the positions of the nerve trunks are compared, it will be found that in the lizard the obturator nerve passes through a foramen in the pubis, and lies far forwards, anterior to the level of the acetabulum, while in the crocodile it lies more posteriorly, having apparently followed the main mass of the adductor musculature as it shrunk back on to the ischium. The flexor tibialis genicularis would appear to have become included in the region supplied by the obturator nerve owing to changes in the position of the nerve, rather than on account of its own migrations.

The muscles of *Sciurus* and *Ornithorhynchus* can be discussed together.

The deep gluteal musculature, represented in Reptiles by the single iliofemoralis, is still represented in Monotremes by only one muscle, the gluteus profundus, but in other Mammals it has become broken up into a gluteus medius and minimus, and sometimes into a gluteus quartus and a pyriformis as well. In Reptiles it has a double nerve supply from the femoral and from the sacral nerve; in the Mammals it has lost one of these and is supplied solely by a sacral nerve, the superior gluteal. The reduction of the double nerve supplies commonly found in Reptiles and Amphibians is usual in Mammals, and is probably due to the concentration of the muscles themselves, so that instead of forming flat wide sheets which attract nerves from two sources, they become rounded off and exert their influence on only one nerve trunk. This process is seen in the Reptiles in the puboischiotibialis and adductor femoris of the crocodile.

The superficial group of gluteal muscles is again represented in most Reptiles by only one muscle, the iliotibialis, with a double innervation from the femoral and from a sacral nerve. In the Mammals the sheet has become divided into several distinct portions. The most posterior is the biceps, which has migrated into the flexor region, and has received a new nerve supply from the nerve to the hamstrings, the n. ischiadicus ventralis. The middle portion is typically subdivided into a gluteus maximus and a femorococcygeus, well seen in *Sciurus*, both supplied by the inferior gluteal nerves from the sacral plexus, reaching them by the old reptilian route. In *Ornithorhynchus* this musculature is highly specialised, for it has completely lost its old attachment to the tibia, represented in typical Mammals by the iliotibial tract, and has become entirely inserted on the upper end of the femur.

The anterior fibres of the superficial gluteal sheet form the tensor fasciae latae, typically developed in *Sciurus*. It was not recognised in *Ornithorhynchus* by Pearson (1926), but it is clearly shown in *Echidna* by Low (1929, "gluteus profundus 2nd pt.") lying superficial to the gluteus maximus ("gluteus superficialis"), and in my specimen of *Ornithorhynchus* appears to be a separate muscle, though it is inserted deep to the gluteus maximus. In most Mammals this muscle is supplied by a sacral nerve, a branch of the superior gluteal which reaches it by a new route, passing deep to or piercing the gluteus medius. In *Ornithorhynchus* also it is chiefly supplied by a similar nerve which pierces the gluteus profundus, but partly by a small, and highly variable (Frets, 1909) branch from the femoral, which reaches it by passing between the rectus femoris and the femur. Possibly this may be the last remnant of the branch from the femoral nerve which, in Reptiles, supplied the anterior fibres of the iliotibialis. The reason for the small size of the twig in *Ornithorhynchus*, and its complete replacement by the superior gluteal nerve in typical Mammals is obscure.

Turning now to the hamstrings, the nerve supply is, in the Mammals as in the Reptiles, preponderantly derived from the n. ischiadicus ventralis, which in *Ornithorhynchus* and in most Mammals passes anterior to the retractor femoralis, just as in Reptiles it passes anterior to the latter's homologue, the flexor tibialis externus. But in *Sciurus* the retractor femoralis arises from a

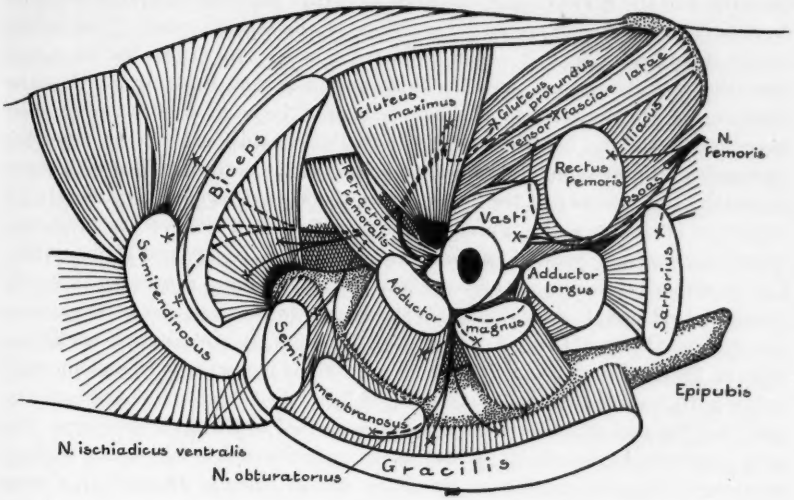

Fig. 5. Long thigh muscles of *Ornithorhynchus*.

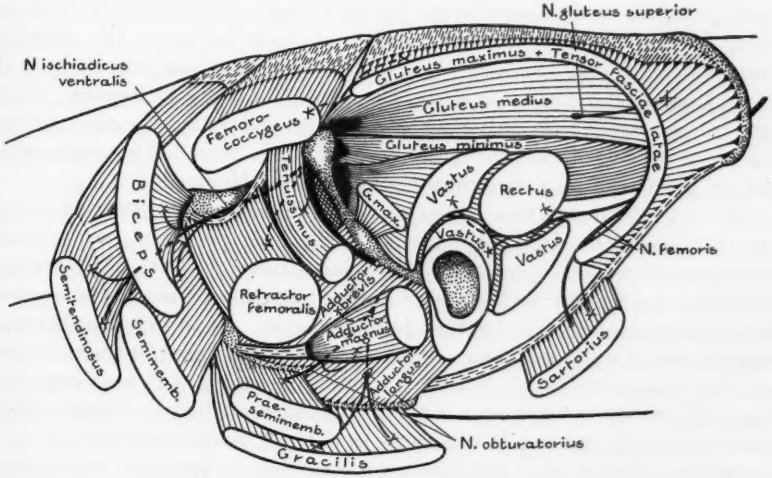

Fig. 6. Long thigh muscles of *Sciurus*.

tendinous arch which stretches from the sacrum to the tuberosity of the ischium, and the nerve crosses this arch medially. So if the caudal origin were to disappear, the nerve would lie posterior to the muscle, while if the ischial origin should disappear, it would lie anterior to it. Hill (1934) has supposed that the first change has actually happened in several Rodents, so that the relations of the muscles to the nerves cannot be used to determine their homologies, and he has thus been led to the conclusion that the retractor femoralis and praesemimembranosus may be the same muscle. But he has not explained the presence of the two muscles together described very clearly by Appleton (1928) in many animals. Even in *Sciurus* I believe the praesemimembranosus to be present as shown in the diagrams (fig. 6, and Haines, 1934, fig. 5), though it has an obturator nerve supply. A more surprising theory is to be inferred from Cunningham's (1882) descriptions and figures of *Thylacinus* and *Cuscus* (Plate III, fig. 1, and Plate IV, fig. 5). It is clear from his figures that in these animals as in *Sciurus* the retractor femoralis ("ischiofemoral") arises both from the caudal vertebrae and from the tuberosity of the ischium, and that the n. ischiadicus ventralis passes medial to the arch so formed. The arch however is described (p. 34) as the sacrotuberous ligament, without comment. If this identification should be correct, then in *Thylacinus*, *Cuscus* and *Sciurus* the nerve takes a course in relation to the ligament similar to that of the pudendal nerve, and quite different from its course in other animals in which the ligament is developed. But the identification of the arch as the sacro-tuberous ligament must be considered very doubtful.

There can be no doubt, however, about the variability of the course of the nerve supply of the semitendinosus. The chief origin of the biceps in all Mammals, including the Monotremes, is from the ischium, while the nerve in *Ornithorhynchus* passes entirely dorsal to it, in other Mammals entirely ventral. If, as I suppose, the biceps is derived from the iliotibialis in Reptiles, which lies entirely dorsal to the n. ischiadicus ventralis, and the semitendinosus from the flexor tibialis inferior, the dorsal position of the nerve in *Ornithorhynchus* is more specialised than its ventral position in most Mammals.

In typical Mammals two hamstring muscles, more or less closely connected together, spring from the ischium and are inserted on the tibia: the semimembranosus and praesemimembranosus, both innervated by the n. ischiadicus ventralis. In *Sciurus* both muscles are well developed, but the praesemimembranosus is supplied by the obturator nerve. In *Ornithorhynchus* two muscles (fig. 5, semimembranosus) are found, but it is doubtful whether they can be compared directly with the semimembranosus and praesemimembranosus. In my specimen one of these muscles is supplied by a branch from the obturator in addition to its supply from the n. ischiadicus ventralis, and in that figured by Frets (1909) both are so supplied. In Reptiles the precursor of both muscles appears to be the flexor tibialis genicularis, which is supplied by the n. ischiadicus ventralis. Thus the nerve supply is more conservative in typical Mammals than in *Sciurus* or *Ornithorhynchus*, where, as in the crocodile,

the change to an obturator supply seems to be due to a relative approximation of the muscles to the nerve trunk which supplies them.

The femoral nerve in Mammals supplies the vasti, the rectus femoris and the sartorius. The vasti are derived from the reptilian femorotibialis, and the rectus from the ambiens, both supplied in Reptiles as in Mammals by the femoral nerve. But the sartorius seems to correspond to the reptilian adductor tibialis, which in typical Reptiles is supplied by the obturator nerve. It is probable that, just as in the crocodile, the migration of the muscle into the extensor region has been associated with a change to a femoral nerve supply.

The adductors are more difficult to compare than the other groups. The single reptilian adductor femoris has a double nerve supply from the obturator and the n. ischiadicus ventralis. Its homologue in Mammals, the adductor brevis, is supplied entirely by the obturator nerve. In *Ornithorhynchus* it is probably represented by one of the short muscles included by Pearson (1926) in her obturator externus and quadratus femoris group, not shown in fig. 5. The puboischiotibialis also has a double nerve supply in Reptiles, but as the gracilis of Mammals only a single obturator supply. The adductor longus of Mammals, and its homologue the flexor intercapitalis anterior of Reptiles, both have an obturator supply. The adductor magnus in *Sciurus* arises by a narrow tendon from the ischium, and is supplied by a branch from the obturator nerve which reaches it passing anterior to the adductor brevis, instead of behind it as in most Mammals. In *Ornithorhynchus* it appears to have become divided into two bellies, between which passes the branch of the obturator nerve which supplies the gracilis and semimembranosus, but its identity must be regarded as very doubtful. In Reptiles the muscle is represented by the flexor intercapitalis posterior, a flexor of the tibia supplied by the n. ischiadicus ventralis, and its change of nerve supply is probably due to its migration into the obturator region where it is closely associated with the other adductors.

In so far as they are new, the detailed evidence for these muscular homologies and changes of nerve supply will be found in my earlier work (Haines, 1934) and is too long to be repeated here. If, however, the identifications are in the main correct, then it can by no means be said that changes of nerve supply are rare, but rather that they are the rule when any extensive migration of muscles has taken place, and that they sometimes occur even when no obvious reason for a change can be found. Now where few migrations have occurred, the homologies of the muscles are clear and are not likely to be disputed, but where the muscles no longer lie in their old positions, any help that the nerve supplies could give in the determination of their homologies would be most valuable. But if the migration itself is likely to lead to a change of nerve supply, it is just in these cases that the evidence of the nerve supply will tend to mislead, rather than to aid, the anatomist.

THE POSTERIOR THIGH MUSCLES OF *TALPA*

In spite of the possibility of variation in nerve supplies, and in the relation of nerves to muscles, these may sometimes be used for the determination of homologies, as has been clearly demonstrated by Appleton (1928) for the posterior thigh muscles. It is my belief that too much reliance on the arrangement of the nerves has led him into error in comparing the muscles of Reptiles

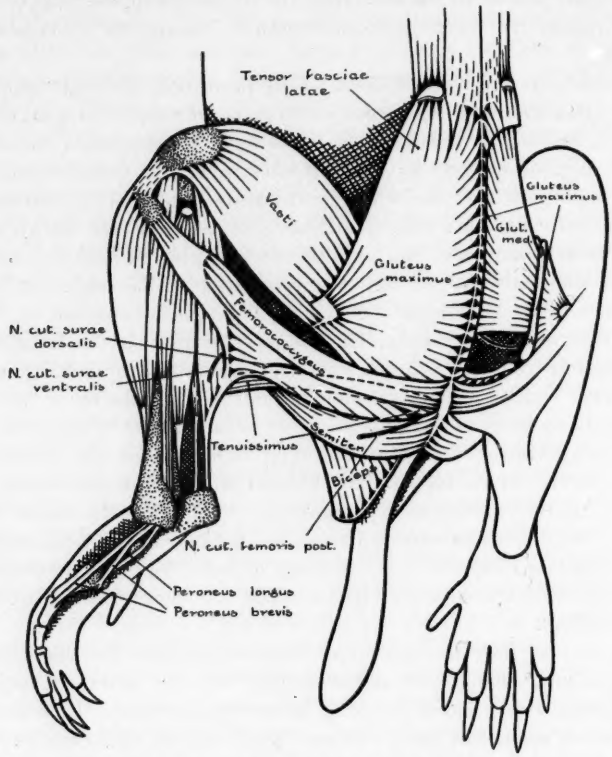

Fig. 7. Posterior thigh muscles of *Talpa*.

with those of Mammals (Haines, 1934), but there can be no doubt of the value of his methods when used for Mammals alone. His identification of the semitendinosus as a hamstring in *Ornithorhynchus*, for instance (fig. 5), was made by a reference to its ventral ischiadic supply; earlier authors had referred it to the gluteal musculature.

The myology of the mole, *Talpa*, one of Appleton's types, demonstrates the value of his technique, for although the thigh has undergone many specialisations, the nerves have remained relatively unchanged. The gluteus

maximus and tensor fasciae latae have, as in *Ornithorhynchus*, entirely lost their attachment to the tibia, and have become inserted on the femur, but the femorococcygeus is quite distinct. Now in typical Mammals it is inserted on the lower end of the femur; in *Talpa* it forms with the biceps a tendinous arch, one end of which is attached to the tibia, and the other with the gastrocnemius to the calcis. Its separation from the biceps, however, is established by its origin from the vertebrae instead of from the ischium, and by the course of

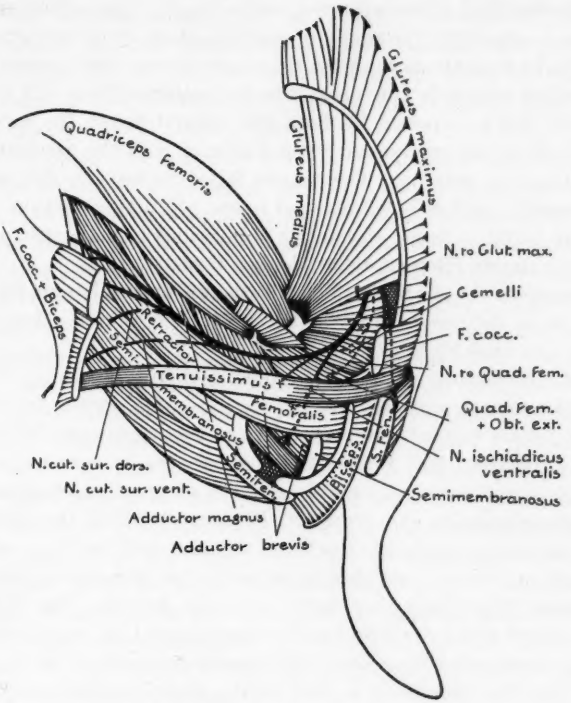

Fig. 8. Deeper thigh muscles of *Talpa*.

the nervus cutaneus surae dorsalis between the two muscles, its usual position in Mammals. The biceps again is atypical in its wide origin from the ischium and in its insertion on the arch, but it retains its usual nerve supply from the n. ischiadicus ventralis, and its usual relation to the n. cutaneus surae dorsalis. The n. cutaneus surae ventralis usually passes to the leg below the lower border of the biceps, between it and the tenuissimus, but it can still be identified in *Talpa*, in spite of its course through the tendinous arch. For the identification of the retractor femoralis the path of the n. ischiadicus ventralis is most important, for in *Talpa* as in most Mammals the nerve passes anterior to the

muscle, serving to distinguish it from the other hamstrings. This relation appears to be constant with a few exceptions, such as *Sciurus*.

Another complex may be unravelled by the use of nerve supplies. There is in *Talpa* no separate muscle clearly identifiable as the quadratus femoris. Leche (1883), in his work on the muscles of Insectivora, described a quadratus femoris in *Talpa* which lay more distally and anteriorly than usual, and was supplied by the obturator nerve, and so could not be homologous with the common mammalian muscle. But it is clear from his figures that this is the muscle here identified as the adductor brevis (fig. 8), lying in its usual position proximal and anterior to the adductor magnus. A study of the nerve supplies, however, shows that the nerve from the sacral plexus which supplies the two gemelli also has a branch which passes to the posterior fibres of a deep muscle sheet. Since this is supplied by both the obturator and the sacral nerves, I believe it to be the representative in *Talpa* of both the quadratus femoris and the obturator externus. These have failed to become differentiated as separate muscles, and so have returned to the reptilian condition, where this musculature forms a single sheet, the puboischiofemoralis externus, with a double nerve supply (Romer, 1922; Haines, 1935).

The examples chosen for detailed discussion have all been from muscles developed from the mesenchyme of the limbs, but muscles developed from myotomes also show changes in nerve supply.

The fin musculature of all Fish is developed from myotomic buds which grow out, two to each segment, into the fins (Goodrich, 1906). In the adult the radial muscles are regularly arranged, two to each segment, alternating with the radial cartilages. But Braus (1910) has shown by electrical stimulation that a single spinal nerve may supply as many as seven muscles, and not only the two belonging to its own segment. He suggested that the radial muscles, though apparently segmental structures, were actually built up each of parts from several myotomes, and that these parts were supplied by the nerves of the segments from which they were originally derived. But Müller (1910) could not accept this interpretation, for it appeared that muscle tissues could not become transposed from one radial muscle to another. So it seems more probable that the polyneury is due to the direct invasion of the muscles belonging to one segment by the nerves of other adjacent segments.

The opposite change, leading to ologoneury, is seen in the serratus posterior superior of *Macacus rhesus* (Ogushi, 1921). In this animal the muscle resembles that of Man in being arranged as a series of slips inserted into the upper ribs (3rd to 6th). But the nerve supply, instead of reaching the muscle as four separate branches from the appropriate spinal nerves, is all derived from the first nerve, from which a single trunk descends to supply all the slips. It is possible that the four slips in *Macacus* are all derived from the first thoracic myotome, but this seems very improbable, for the muscle as a whole bears the closest resemblance to that of related monkeys where the nerve supply is normal.

Two similar examples of oligoneury have been fully discussed by Addens (1933). The development of the electric organ of the star-gazers was worked out by White (1918), who found that it was supplied entirely by the oculomotor nerve, although it was built up from tissue belonging to the first three segments. In the tails of Mammals Schumacher (1910) found that, although the muscles belonged to several segments, a point confirmed embryologically by Butcher (1929), they are supplied by only a few nerves, joined together to form a trunk. Schumacher supposed that the reduction of the nerves took place during the evolution from Reptiles to Mammals, as he derived the mammalian muscles from the reptilian ilio- and ischiocaudalis. But it seems more probable that they are actually derived from the caudofemoralis in which the nerve supply is already reduced to a few post-sacral nerves, which join to form a trunk in the muscle (Gadow, 1882; Haines, 1935).

Addens' examples of oligoneury in myotomic muscles whose development is known are important as showing that there can be a complete change of nerve supply to a muscle, not only in the peripheral course of the nerve, but also in its central origin. In the cranial nerves Addens has shown clearly that complete changes of nerve supply are rare while central anastomoses are the rule, but in the limbs complete changes are probably frequent. Several examples have been quoted for instance to show that a muscle may be supplied by the obturator nerve in one animal and by a sacral nerve in another, and there is no reason to suppose that these are not simple examples of complete change of nerve supply. For changes in the nerve roots supplying muscles certainly occur (Bardeen, 1907; Frets, 1909), and if when this happened the nerve bundles to the muscles still arose from the original group of ganglion cells, they would have to ascend or descend in the cord to join the roots with which they now emerged. But in the brachial and lumbosacral regions of the cord such bundles have not been found, and it seems improbable that any specific relation exists between particular muscles and particular nerves. Experimental studies on the innervation of the brachial muscles of urodeles have led to the same conclusion (Detwiler, 1934; Detwiler and van Dyke, 1934).

My thanks are due to Prof. C. M. West for the interest he has taken in this work, and for the loan of material.

SUMMARY

1. It was the opinion of Fürbringer that the nerve paths were established as protoplasmic bridges, which persisted from the earliest divisions of the ovum, and which could not be broken however much the muscles might wander, so that the nerves could be used as infallible guides in the determination of muscular homologies. Apparent changes of nerve supply from one nerve trunk to another were due to the vicarious development of muscles which were not homologous.

2. It is here argued that muscles may be homologous in spite of changes of nerve supply, and that the nerve supplies are inconstant in phylogeny in

their central origins, in the plexuses from which they are derived, and in their courses. The adductor tibialis, for instance, is supplied by the obturator nerve in lizards and by the femoral nerve in crocodiles, and the flexor tibialis genicularis by the n. ischiadicus ventralis and by the obturator. The n. ischiadicus ventralis again lies ventral to the biceps in most Mammals but dorsal to it in *Ornithorhynchus*.

3. This inconstancy is found both in cranial and in spinal nerves, and in nerves supplying muscles developed from myotomes as well as those developed independently in the limbs.

4. Nevertheless a study of the nerves may be very useful in the determination of homologies, particularly between the muscles of animals belonging to a single class, such as the Mammalia, but has led to error in the comparison of the muscles of Mammals with those of Reptiles.

5. Changes in nerve supply are usually associated with a migration of the muscles, so that these come to lie nearer the nerve trunks which give them their new supplies.

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THE EVOLUTION OF THE HUMAN FOOT, WITH ESPECIAL REFERENCE TO THE JOINTS

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The current divergence of views concerning the evolution of the human foot is due largely to a lack of accurate knowledge concerning the functioning of the ape foot, and a concentration of attention on morphological features less intimately concerned with the action of the foot than are the joints. By means of an apparatus for studying the instantaneous distribution of pressure in the sole of the foot, we have been able to make an accurate comparison between the method of function of the chimpanzee foot and that of man (Elftman and Manter, 1935). In the present paper we shall consider especially the joints of the chimpanzee foot in comparison with Man, with regard to their bearing on the evolution of the human foot.

THE TRANSVERSE TARSAL JOINT AND THE LONGITUDINAL ARCH

The transverse tarsal joint (mid-tarsal, Chopart's) lies between the calcaneus and talus on one side and the cuboid and navicular on the other (figs. 1, 3). Movement in this joint allows the forepart of the foot, which is quite rigidly attached to the navicular and cuboid, to move with respect to the calcaneus and talus about an axis which is shown in fig. 2. These movements we shall refer to as plantar-flexion and dorsi-flexion about the transverse tarsal joint. Since the axis of the joint in the normal position of the human foot is inclined at a considerable angle to the horizontal, plantar-flexion with the calcaneus held immovable results in adduction of the forepart of the foot. Dorsi-flexion results in abduction.

When movement takes place in the transverse tarsal joint with the foot on the ground, the tuber calcis and the anterior part of the foot must remain in contact with the substratum. Under these circumstances dorsi-flexion about the transverse tarsal joint results, not in abduction of the forepart of the foot, but in a lowering of the joint and a flattening of the foot.

In the chimpanzee the transverse tarsal joint is freely movable, while in the human foot with a well-developed longitudinal arch the joint is relatively immobile. The human foot with its longitudinal arch (fig. 1B) displays the same relationships between the forepart of the foot and the calcaneus and talus that we find in the chimpanzee foot when it is plantar-flexed about the transverse tarsal joint (fig. 1A). We are justified in concluding that in the human foot the transverse tarsal joint has become fixed in a plantar-flexed position.

Additional evidence for this concept is to be found in the prevalence of mobility in the transverse tarsal joint in flat-footed humans. It seems likely that mobility in the transverse tarsal joint, rather than being a result of flat-footedness, is more often a cause.

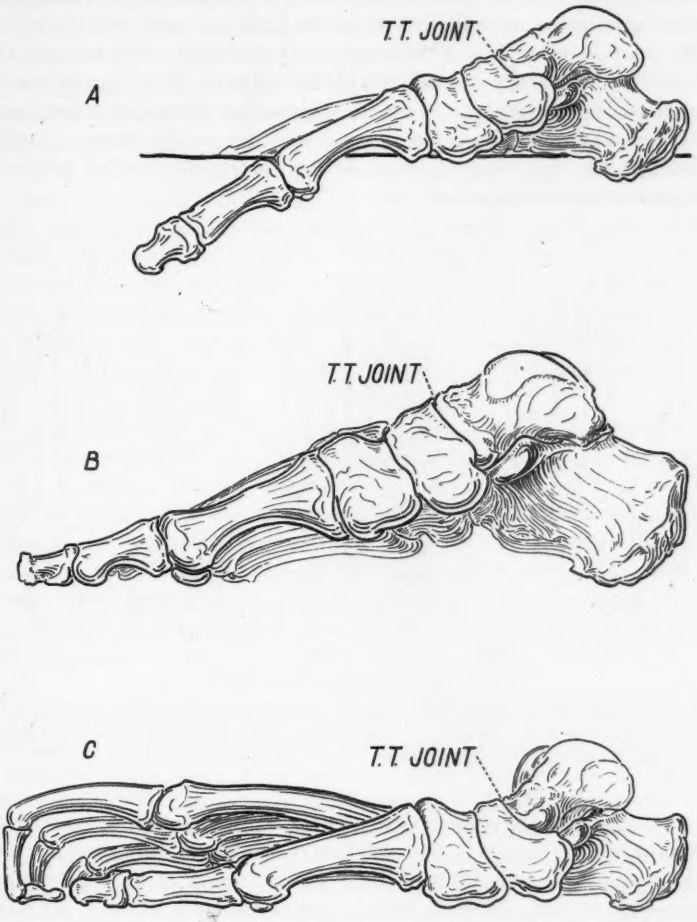

Fig. 1. Chimpanzee and human feet in medial view. A, chimpanzee foot in an arboreal position; plantar-flexion about the transverse tarsal joint. B, human foot on the ground. C, chimpanzee foot on the ground: pronation with dorsi-flexion about the transverse tarsal joint. *T.T.* transverse tarsal joint.

The evolution of the longitudinal arch in Man is consequently due to an immobilisation of the transverse tarsal joint in a plantar-flexed position. To provide a more complete explanation involves the determination of the

mechanism of immobilisation. This cannot be done at present. It might be due to a shortening of such connective structures as the plantar aponeurosis, the long plantar ligament and the plantar calcaneo-navicular ligament. It could also be accounted for by changes in muscle action. It will be impossible to decide this issue until we are able to settle by experiment the question as to whether ligaments or muscles determine the limits of movement in the joints.

The position of the axis of the transverse tarsal joint which we have determined differs from the position given by Fick (1911). There can be no doubt concerning the position of the axis in the chimpanzee. Since our determinations on mobile and juvenile human feet agree with the results in the chimpanzee, we feel that Fick must have been misled by movements in other parts of the foot than the transverse tarsal joint.

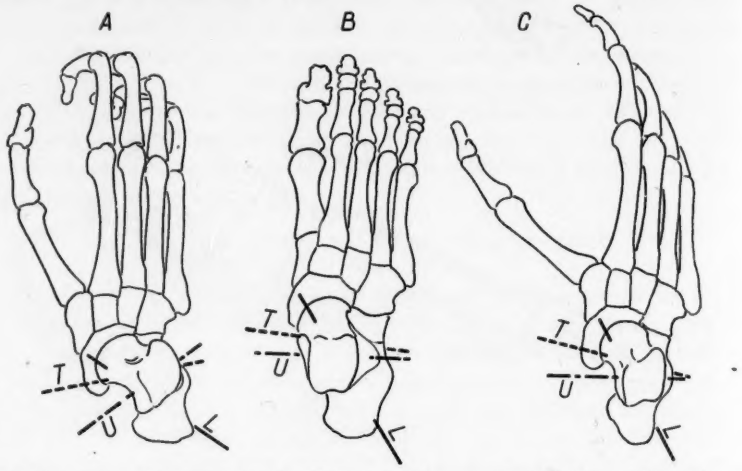

Fig. 2. Chimpanzee and human feet in dorsal view. A, chimpanzee foot on the ground. B, human foot on the ground. C, chimpanzee foot plantar-flexed about the transverse tarsal joint, as in fig. 1A. *T*. axis of transverse tarsal joint. *U*. axis of upper ankle joint. *L*. axis of lower ankle joint.

The importance of plantar-flexion or inversion about the transverse tarsal joint as one of the factors in the evolution of the longitudinal arch has already been stressed by Keith (1923, 1929). The extension of his theory so as to assign prime importance to the tibialis anterior as an invertor of the foot has, however, been rendered doubtful by the studies of Fick (1911, 1931) and by our own determination of the position of the axis of the transverse tarsal joint. The tibialis anterior may well be a postural muscle, intimately correlated with the evolution of the upright posture of man, due to its action about the upper ankle joint.

Attempts have been made to explain the evolution of the longitudinal arch on the basis of changes in the position of the talus. It is true that plantar-

flexion in the transverse tarsal joint, with the heel and anterior support of the foot in contact with the ground, does tend to bring the talar head more directly above the calcaneus, especially since this plantar-flexion is usually associated with inversion about the lower ankle joint. But the change in position of the talar head is a consequence, not a cause, of longitudinal arch production. The arch is a subtalar structure, depending on the relationship of the calcaneus to the anterior part of the foot.

Weidenreich (1921) recognises that plantar-flexion about the transverse tarsal joint has occurred in the evolution of the longitudinal arch, but considers this as being subsidiary and subsequent to the upward inclination of the calcaneus. It seems obvious to us that the upward inclination of the calcaneus is a necessary concomitant of fixation of the transverse tarsal joint in the plantar-flexed position and cannot be considered as a cause of the plantar-flexion. Certainly Weidenreich's argument that in Man the weight of the body is concentrated on the calcaneus and thus is responsible for its tilt is not substantiated by our researches on the path of the resultant of pressure in the foot (Elftman and Manter, 1935). In the chimpanzee the resultant lingers for a longer time in the tarsal region than it does in Man, but the chimpanzee gives no evidence of a calcaneal tilt.

Recognition of the evolution of the longitudinal arch as having been due to plantar-flexion about the transverse tarsal joint serves also to explain the fact that in the human foot the metatarsals are in line with the long axis of the calcaneus, while in the ape foot, as seen resting on the ground (fig. 2A), the metatarsals diverge laterally from the long axis of the calcaneus. If the chimpanzee foot is plantar-flexed about the transverse tarsal joint, with the heel and anterior support of the foot still in contact with the ground (fig. 2C), it will be found that the metatarsals in their new position are parallel to the long axis of the calcaneus.

THE LOWER ANKLE JOINT

In the ankle there are two joints about which movement is possible. In the upper ankle joint (talo-crural), the tibia can rotate backwards or forwards over the trochlea of the talus. It is the lower ankle joint (subtalar), however, to which we shall first direct our attention. In this joint the articulation is between the talus on one side and the calcaneus, cuboid and navicular on the other. This gives rise, according to the usual description, to the possibility of gliding movements in the joint. A more illuminating concept is that of Dönitz, modified by Fick (1911), who demonstrated that, in spite of the bewildering congeries of articular surfaces involved, the movement in the lower ankle joint can be quite accurately described as rotation of the entire subtalar portion of the foot about a line, the compromise axis of the lower ankle joint. The position of this axis is shown in figs. 2, 3, 4 and 5. When looking at the right foot from in front, a clockwise rotation of the foot about this axis would give rise to eversion, a counter-clockwise rotation to inversion.

It is possible to tell whether a foot is being held in an inverted or everted

position by noting the relations of the talus to the calcaneus, since the joint surface passes between these bones. The talus and calcaneus of the chimpanzee are represented in fig. 3A in the relative positions they occupy when on the ground, but with the calcaneus tilted so as to make comparison with the other figures easier. Fig. 3C shows these bones in the positions they occupy in the arboreal position of fig. 1A. It is obvious that when on the ground the chimpanzee foot is everted about the lower ankle joint, while in the arboreal position illustrated a moderate degree of inversion is present. If the human relationships (fig. 3B) are now compared with the two figures of the chimpanzee, it is apparent that the usual human position in standing resembles that of the chimpanzee when moderately inverted.

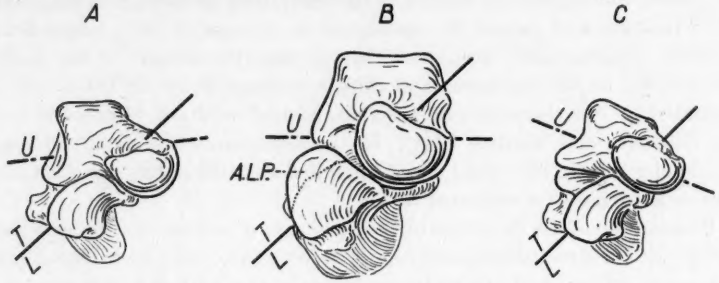

Fig. 3. Anterior view of the talus and calcaneus, with the plane determined by the axes of the transverse tarsal and lower ankle joints perpendicular to the plane of the figure. A, chimpanzee, showing the relationships of the bones in the terrestrial position of fig. 1C, but with the whole figure rotated outward so as to bring the calcaneus into a position congruent with that of fig. 3C. B, human. C, chimpanzee, plantar-flexed about the transverse tarsal joint with accompanying inversion about the lower ankle joint, as in fig. 1A. *U.* axis of upper ankle joint. *T.*, *L.* axes of transverse tarsal and lower ankle joint. *A.L.P.* antero-lateral process, its extent roughly indicated by the dotted line.

The normal human foot can never achieve as highly everted a position as that shown to be the common terrestrial position of the chimpanzee. One of the reasons for this is found by an inspection of the anterior portion of the calcaneus of Man. Both in the anterior and lateral views of fig. 5, and in fig. 3B, there can be seen a bony process, which we shall call the antero-lateral process of the calcaneus, which prevents extensive movements of eversion of the talus with respect to the calcaneus. No indication of this process can be found in the calcaneus of either chimpanzee or gorilla. It furnishes one of the most easily recognisable diagnostic characteristics of the human type of calcaneus.

RELATIONSHIP OF THE AXES OF THE TRANSVERSE-TARSAL AND LOWER ANKLE JOINTS

The articulation between the talus and the navicular presents a peculiar problem, since movement in this articulation may take place either about the axis of the lower ankle joint, about the axis of the transverse tarsal joint, or

about both axes simultaneously. This would not be possible if the articular surface were not essentially spherical, with the two axes mentioned intersecting at the centre of curvature of the sphere. Since the two axes do intersect at this centre of curvature, free movement can take place simultaneously in the lower ankle joint and in the transverse tarsal joint.

The extent of the articular surface on the head of the talus is intimately correlated with the degree of movement possible. The greater range of eversion possible in the chimpanzee is correlated with the extension of the articular surface (fig. 4) further posteriorly on the lateral side in the chimpanzee than in Man.

THE UPPER ANKLE JOINT

The talo-crural or upper ankle joint allows movement between the tibia and fibula, acting together, on one side, and the talus on the other. We should consequently expect that in the evolution of the human upright posture, with the knees held near the midplane of the body during movement, there would be a remodelling of the tibia, fibula and talus. The differences between the chimpanzee and Man in the tibia and fibula have been adequately treated by former investigators.

A comparison of the talus of Man with that of the chimpanzee shows that the trochlea in the human has been rotated upon the body of the talus. This rotation is clearly seen in the dorsal views of the bones (fig. 4) by concentrating attention upon the angle between the neck of the talus and the axis of the upper ankle joint. This angle is larger in Man than in the chimpanzee. This difference in angle has been noted by many previous workers. They, however, have interpreted it as indicating a shift in position of the neck of the talus. That the neck has not changed can be seen by noting its relations to the articular surfaces as seen in ventral view. Further confirmation of our interpretation that it is the trochlea which has undergone rotation emerges from a comparison, in posterior view, of the relation of the trochlea to the posterior calcaneal articular surface.

The medial border of the trochlea is higher in Man than in the chimpanzee, as can be seen in the anterior and posterior views (fig. 4). This is also correlated with changes in the tibia.

THE METATARSO-PHALANGEAL JOINTS

That the five metatarso-phalangeal joints are differently oriented in Man than in the chimpanzee and gorilla has been noted by many investigators, including Morton (1922) and Weidenreich (1921). In fig. 6 the heads of the metatarsals are shown in projection upon a vertical plane. It is apparent that in the human there is not a well-developed transverse arch in this region, as there is in the chimpanzee. In a previous paper (Elftman, 1934) the fact that there is no concentration of pressure, such as one would expect at the ends of a functional arch, was taken to be an indication of a lack of a transverse arch functional in weight-bearing in this region of the human foot. It is possible for

the human foot to distribute its pressure over this entire region of the ball of the foot when the heel is lifted, because of the disposition of the axes of the five metatarso-phalangeal joints. In the chimpanzee this is not possible. The axes of the fifth and first metatarso-phalangeal joints are sharply inclined to the ground. It is consequently impossible for the foot as a whole to bend in a vertical direction in this region. When the heel of the chimpanzee and then the transverse tarsal joint are lifted, pressure is transferred not to the region of the metatarsal heads but to the toes.

The evolutionary changes which have resulted in the human condition have been described as torsions of the metatarsals, the first in one direction, the four lateral ones in the other. It is unquestioned that the distal portions of the metatarsals have undergone changes in which the bases have not participated.

THE JOINT AT THE BASE OF THE FIRST METATARSAL

The opposability of the hallux in the apes contrasts greatly with its permanently adducted position in Man. Since this condition is apparent externally it has been seized upon as the prime difference between the ape and the human foot. There is no doubt but that in the evolution of the foot other features are more important, but it is true that an adducted hallux provides a stronger anterior pillar for the longitudinal arch than would an abducted hallux.

The articular surface on the first cuneiform of the chimpanzee is strongly convex and faces medially. In Man the articular surface is more nearly flat and faces more anteriorly. Some specimens of human cuneiform do, however, possess a markedly convex surface, the axis of curvature being oriented as in the chimpanzee, but the articular surface faces forward. There is no doubt whatever that the human condition could have evolved from that found in the apes. Detailed evidence for this has been put forward by Schultz (1930).

THE BONES OF THE FOOT

The evolutionary changes in the joints have inevitably been accompanied by changes in the bony elements. It is advisable, therefore, to compare briefly the more essential features of the bones of the human and chimpanzee feet.

The talus

In comparing the talus of Man with that of the chimpanzee it is necessary that the two bones be oriented in similar fashion. The usual method is to use the trochlea as a basis of orientation. This is highly unfortunate, since we have seen that the trochlea changes in the course of evolution. We shall therefore orient the two bones with the axes of the lower ankle joints parallel and the ventral articular surfaces in congruent positions.

When this is done, many of the differences which have been described as existing between the two bones are seen to be due to faulty orientation. The general similarity of the ventral articular surfaces (fig. 4) is apparent. It is true that the radius of curvature of these surfaces is shorter in the chimpanzee than

in Man. There is difficulty in estimating the importance of this, however, since the radius of curvature of these surfaces is shorter in small human feet than in large ones.

The rotation of the trochlea with respect to the rest of the bone and the increased height of the medial border of the trochlea in Man have already been discussed. The latter feature is of importance in studying the head of the talus. In the anterior view of fig. 4, it is noticeable that the greatest length of the talar head in the human makes a larger angle with the dorsal surface of the trochlea than it does in the chimpanzee. This has been interpreted as due to a torsion of the talar head. It is obvious, however, that here, again, it is the trochlea and not the head of the talus that has changed. The difference in

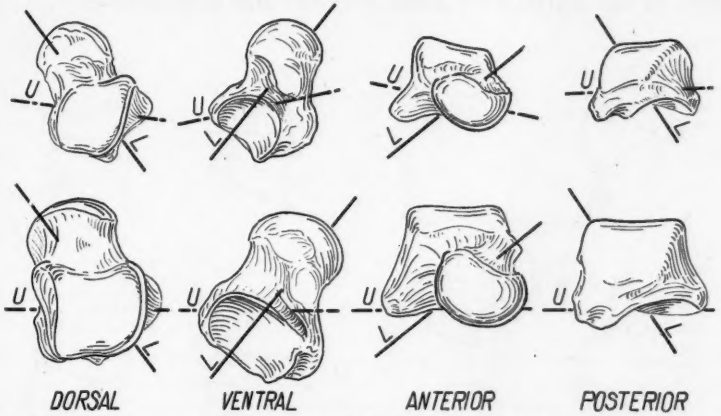

Fig. 4. The talus of man and chimpanzee oriented with the axes of the lower ankle joints parallel. Upper row: chimpanzee. Lower row: human. *U*. axis of upper ankle joint. *L*. axis of lower ankle joint.

extent of articular surface on the head of the talus when the chimpanzee is compared with Man contributes to this apparent torsion. But this we have already found to be due to greater possibilities of eversion in the chimpanzee, not to a change in the axis of the joint.

The variations in the anterior extent of the trochlear surface in primitive tribes of Man have been extensively discussed by anthropologists and need not be considered here.

The calcaneus

When the calcaneus is oriented by means of the axes of the lower ankle joint and the transverse tarsal joint (fig. 5), there is a striking similarity between the chimpanzee and human bones. One of the chief differences to be noted is the appearance in Man of a new process, the antero-lateral process of the calcaneus, which may be seen in anterior, lateral and dorsal views. The significance of this in limiting eversion in the lower ankle joint has been mentioned.

The similarity in contour of the facets for articulation with the cuboid is worthy of mention. Except for the additional area due to the antero-lateral process, the human facet illustrated is characteristically like that of the chimpanzee. In some other human calcanei examined, the transverse groove which indents this facet is not so well developed. It is likely that variations in this groove are correlated with the variability in the mobility of the transverse tarsal joint.

The shape of the tuber calcanei, seen in posterior view (fig. 5), is extremely variable in Man. It is frequently quadrate in appearance, but varies from this condition to one of ovate contour resembling more closely the chimpanzee. The calcaneus of Man is relatively longer and stouter than that of the chimpanzee. In this character the gorilla resembles Man more closely.

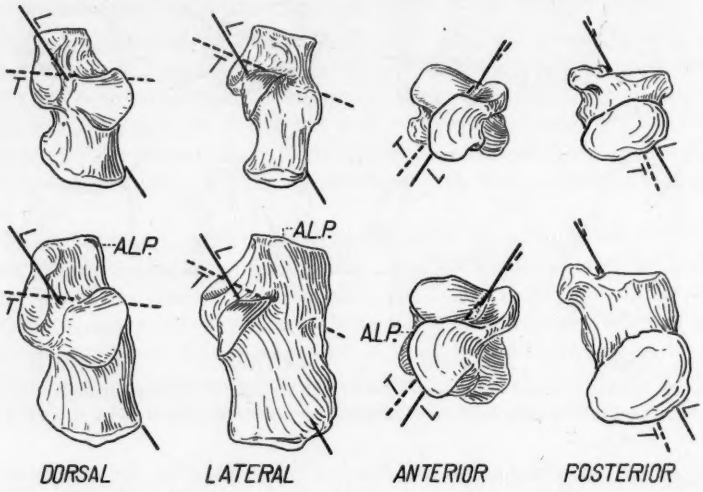

Fig. 5. The calcaneus of Man and chimpanzee oriented with the axes of the lower ankle joint parallel. Upper row: chimpanzee. Lower row: human. *L.* axis of lower ankle joint. *T.* axis of transverse tarsal joint. *A.L.P.* antero-lateral process of calcaneus.

The sustentaculum tali has received the attention of previous investigators, some of whom have felt that its position in the human required extensive explanation. A study of the anterior and posterior views of fig. 5 reveals no significant difference in the relationship of the sustentaculum to the rest of the calcaneus between the chimpanzee and Man. Whatever differences it has with respect to the substratum as the foot is used must be due to the position of the entire calcaneus, not to any change in the sustentaculum alone.

The trochlear process or peroneal tubercle is exceedingly variable in Man. In the chimpanzee illustrated this process is more highly developed than it is in the human, but in another specimen of chimpanzee calcaneus it approaches closer to the human condition.

Other tarsal elements

The navicular, cuboid and second and third cuneiforms of Man are longer than those of the chimpanzee, when compared with the combined length of tarsus and metatarsus. This lengthening is balanced to a certain extent by the shortening of the four lateral metatarsals. The cuboid of Man has a larger surface for articulation with the calcaneus, covering in part the anterior face of the antero-lateral process of the calcaneus. The differences in relative proportions of the second and third cuneiforms cannot be discussed here; they may be correlated with a more important feature, the shape of the first cuneiform. The surface on the first cuneiform which articulates with the first metatarsal has already been discussed.

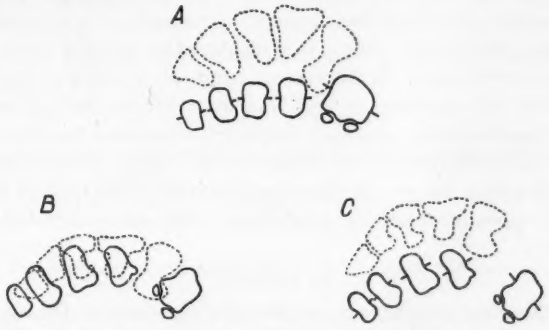

Fig. 6. The heads and the articular surfaces of the bases of the metatarsals, projected upon a vertical plane. The articular surfaces of the bases are shown in dotted lines. A, human. B, chimpanzee in normal terrestrial position. C, chimpanzee with foot plantar-flexed about the transverse tarsal joint.

Metatarsals and phalanges

In addition to the torsion of the heads of the metatarsals with respect to their bases, there is another difference of importance between Man and the chimpanzee. This is the shortness of the four lateral metatarsals and their phalangeal series in the human. Schultz (1930) has demonstrated that in the course of evolution the first metatarsal and its phalanges have undergone no marked change in length, when measured with relation to the rest of the foot, but that there has been an actual reduction of the four lateral metatarsals and their phalanges, especially the second phalanges. It is significant that in the gorilla, which is more terrestrial in its habitat than is the chimpanzee, the lateral phalanges are shorter.

THE MECHANISM OF FOOT EVOLUTION

The contribution of the present paper lies chiefly in a clarification of the structural differences between the ape foot and the human. An analysis of the mechanism of foot evolution must interpret these structural differences from two points of view: first, the changes in the developmental process which lead

to differences in adult structure; and secondly, the differences in function of the adult structures which determine whether they shall be judged fit or unfit by the environment in which they struggle for existence.

The first of these problems has been studied by numerous investigators, notably in recent years by Straus (1927). It would be well worth while to re-analyse the descriptive embryological data available in terms of the structural changes noted in the present paper, with a view to finding, not evidence for recapitulation, but some clue to the developmental factors involved. We fully realise that an analysis into such factors as differential growth rates is but a preliminary step toward a definitive analysis in more fundamental terms, such as genic differences.

The second problem, that of selection by the environment for procreation of those structures which are functionally advantageous, is in some ways more easily studied. We can determine, in a fashion, the changes which have taken place in the environment. In the case at hand, we at least know the environments in which the chimpanzee and Man live. We can tell, by studying the structures experimentally, why they have survival value in the forms now living. If we knew more definitely the environments which surrounded the stages intervening between the ape ancestor and Man, we could make a fair estimate as to which types of structure would have had survival value during this critical period.

SUMMARY

The fundamental similarity in architecture demonstrated in the comparison of the foot of the chimpanzee with that of Man leaves no doubt as to the evolution of the human foot from that of an ape. But superimposed upon this fundamental similarity are important differences which allow us to make the following list of the most important changes which the foot has undergone in its evolution from ape to Man:

1. Stabilisation of the transverse tarsal joint in a position of plantar-flexion. This accounts for the longitudinal arch and partially for the alignment of the lateral metatarsals with the long axis of the calcaneus.

2. Prevention of extensive eversion in the lower ankle joint, partly by the appearance of a new process, the antero-lateral process of the calcaneus.

3. Rotation of the trochlea of the talus with respect to the body of the bone, and raising of the medial border of the trochlea, correlated with the changed position of the tibia in upright locomotion.

4. Torsion of the metatarsal heads, allowing flexion in the metatarso-phalangeal joints perpendicular to the ground. This eliminates the anterior transverse arch as a functional factor and allows the distribution of pressure over the ball of the foot.

5. Permanent adduction of the hallux, forming a strong anterior support for the longitudinal arch.

6. The following changes in proportion: shortening of the four lateral metatarsals and their phalangeal series; increase in relative length of the

cuboid, navicular and first and second cuneiforms; and increase in stoutness and length of the calcaneus.

Although the gorilla resembles Man more closely than does the chimpanzee in the relative shortness of the lateral digits, it shows no indication of the more fundamental changes, such as those in the transverse tarsal joint, which are essential for the development of the human condition.

The fact that the human foot, adapted as it is for walking on the ground, bears a closer resemblance to the ape foot as used in arboreal than in terrestrial locomotion may be regarded as another evidence of Man's arboreal ancestry. It would also suggest that the essential features of Man's foot were acquired at an early stage of his terrestrial existence, rather than after long apprenticeship on the ground. It would be perfectly possible, however, for a human type of foot to evolve from either the chimpanzee or gorilla foot as now constituted, since the structures which, by modification, can give rise to the human structure, are still intact.

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NOTES ON THE DEVELOPMENT OF THE PREPUCE

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INTRODUCTION

The differentiation of the prepuce was described by Retterer and Neuville (1) to be brought about by a "glandulo-preputial epithelial invagination of cells" which, towards the end of intra-uterine life, breaks down to form a slit-like space between the preputial skin and the glans penis. The breaking down of the epithelial cells, according to them, is due to the sudden enlargement of the glans at this time, the degenerating cells first forming isolated intra-epithelial lacunae which then run together and become the glandulo-preputial space.

Berry Hart (2) in an earlier paper had described the process differently. The prepuce, according to him, grows forward to cover a previously naked glans penis. He states that, in his opinion, the prepuce does not grow forward as a free unattached sheath, but that the fold rolls forward to become inverted "so that what was superficial is now deep, facing the glans surface". He then states that the glans and the fold become blended and continuous as a tissue, the future preputial space being filled with epidermal cells, the central of which correspond to the superficial epidermal layers. These central cells, Berry Hart states, "desquamate", and by this desquamation produce the preputial space. Berry Hart studied only two stages of development ($2\frac{1}{2}$ months foetus and at birth), but he believes, he says, that the view which he advanced "may be inferred with some safety from the earlier state".

This account of the development of the prepuce appears to have been generally accepted, and since Berry Hart's time has been reproduced in most text-books of embryology.

Frazer (3) in his *Manual of Embryology* follows it. He writes: "About the end of the second month a low ridge appears round the coronary sulcus marking off the glans. This ridge is the beginning of an upgrowth of the superficial tissues proximal to the sulcus, which continuing its growth leads to the formation of the prepuce. As it grows the epithelial cells covered by it form a solid layer deep to it: they break down subsequently, leaving the structure applied to the underlying glans without any adhesion between their surface other than remnants of the epithelial attachments." Neither Berry Hart nor Frazer offers any explanation of the mode of formation of the frenulum.

A different account of the development of the prepuce was given in 1921

by Spaulding (4). He describes the prepuce to be formed from a pair of swellings at the sides of the urethral opening, which fusing over the dorsum of the shaft of the penis, form a flattened ridge of skin whose distal margin envelops the proximal margins (corona glandis) of the penis. The subsequent growth of this ridge of skin, Spaulding states, results in the progressive inclusion of the glans by the distally migrating fold of skin until in foetuses of 100 mm. C.R. lengths the originally naked glans is entirely covered. Spaulding in his paper then admits that his observations "are based only upon the external examination of the genitalia of older embryos, and have not been confirmed by histological study." "For these reasons", he continues, "they must be considered merely as suggestions of what apparently takes place, and may later be corroborated or contradicted."

The purpose of the present paper is to supply the histological studies omitted by Spaulding, and to discuss how far they support or contradict his work, and to compare his conclusions, which broadly agree with those of Berry Hart, with those of Retterer and Neuville.

MATERIAL

The material used in these studies was entirely human. Serial sections 10 μ in thickness were cut in the sagittal plane of the external genitalia of male foetuses of 40 mm., 70 mm., 100 mm. and 170 mm. C.R. length, and a second series of sections from specimens of the same size were cut at right-angles to the long axis of the penis. These sections were stained with Erlich's haematoxylin and eosin, and wax-plate reconstructions were made from them.

RESULTS

The penis at the 40 mm. stage presents a naked rounded extremity on the surface of which is a thin layer of desquamating epithelial cells. The terminal portion of the penis is demarcated from the shaft by a shallow groove, and a low ridge of epithelial tissue is present just behind this groove (text-fig. 1). The cavernous tissue of the penis is represented by a clearly marked condensation of mesodermal tissue which is stained more deeply than the surrounding tissue.

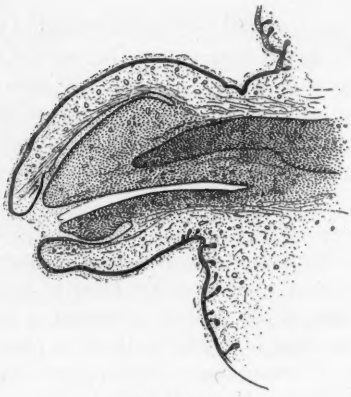
A reconstruction of the penis at this stage shows that the ridge of epithelial tissue behind the groove which demarcates the glans from the body of the penis does not uniformly surround it in the form of a circle (text-fig. 5 A), but is rather disposed in an oblique direction, radiating out on either side of the penis from the point of the urethral opening placed well back on the ventral surface of the organ. It appears as a kind of "hood" on the dorsum, gradually becoming less well marked as its ventrally continued folds approach the urethral opening.

The penis of the 70 mm. stage (text-fig. 2) shows a more definite condensation of the mesodermal tissues and a beginning of differentiation into cavernous tissue. A thick layer of desquamating cells is spread over the surface of the

Text-fig. 1.

Text-fig. 2.

Text-fig. 3.



Text-fig. 4.

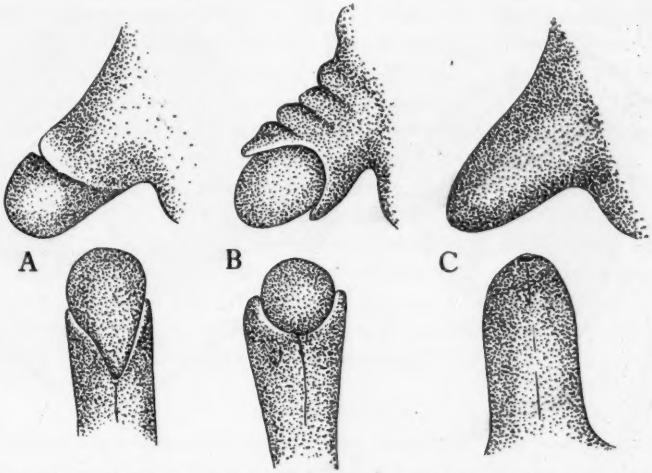
Text-fig. 1. L.S. Penis of human foetus 40 mm. C.R. length. Note the ridge of ectoderm behind the corona and the glans, and the thin layer of desquamating cells on the surface.

Text-fig. 2. L.S. Penis of human foetus 70 mm. C.R. length. Note the dorsal ectodermal tissues thrown into loose folds, and the distribution of the desquamating cells.

Text-fig. 3. L.S. Penis of human foetus 100 mm. C.R. length. The prepuce is here seen "overflowing" the glans and enclosing a layer of desquamating cells between it and the glans.

Text-fig. 4. L.S. Penis of human foetus 170 mm. C.R. length. This section shows the prepuce covering the whole of the glans, with the layer of desquamating cells connecting the two. Note the "plug" of desquamating cells closing the opening of the urethra.

glans, and is in continuity with the epitrichial[1] layer of the general skin. The skin on the dorsal aspect of the penis is thrown into folds, as if it were developing at a much more rapid rate of growth than the differentiating mesoderm into cavernous tissue. At the same time it appears as if flowing over the dorsum of the glans which at this stage is clearly separated from the shaft of the penis by a well-marked sulcus. On the ventral aspect of the organ the urethral opening is placed farther towards the extremity of the penis than in the 40 mm. stage (text-fig. 5 B), and the folds of ectodermal tissue passing downwards and forwards, from the dorsal prepuce, gradually become less well marked as they approach the urethral opening.



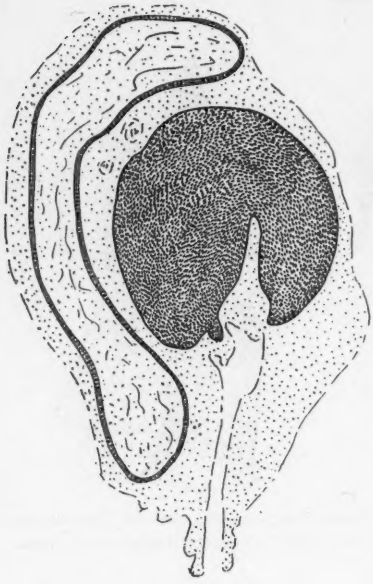
Text-fig. 5. Shows the lateral and ventral aspects of wax-plate reconstructions of three stages in the development of the prepuce: A, 40 mm. C.R. length; B, 70 mm. C.R. length; C, 170 mm. C.R. length.

The genital area of the 100 mm. stage (text-fig. 3) shows a still further differentiation in form and structure. The ectodermal tissue of the body of the penis has the appearance of flowing over the clearly differentiated glans. The layer of desquamating cells on the surface of the glans is irregularly thickened, the ectodermal tissue of the developing prepuce encloses a layer of the desquamating cells, and the prepuce more nearly encircles the glans. The

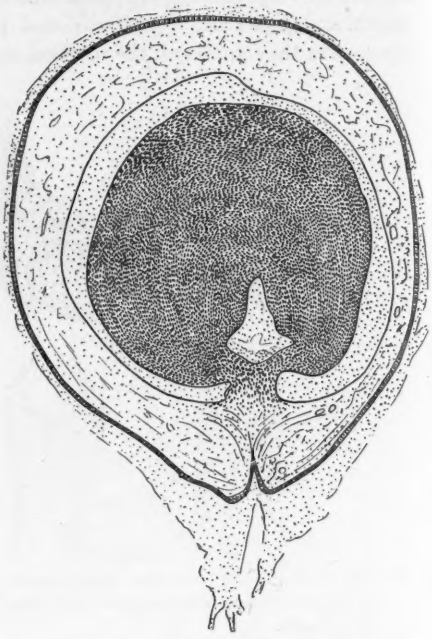
[1] The term "epitrichial" is an unfortunate one, as it implies the presence of hair, and this layer is found in the human embryo at a stage long before there is any trace of the epithelial cords from which the hair is developed. In addition, it is developed to an even greater extent in those areas of the skin, as for example the prepuce, in which there is a complete absence of hair follicles than in the hair bearing areas. This growth of the epitrichial layer in such areas suggests that there may be some connection between it and the absence of hair development; that is in those regions where the epithelial hair shafts do not develop, the ectodermal growth expresses itself in a generalised proliferation as against the specialised growth of the anlagen of the hair.

wax-plate reconstruction shows the urethral opening in a position still more advanced towards the tip of the glans than in any of the earlier stages.

At the 170 mm. stage (text-fig. 4) the prepuce completely encloses the whole surface of the well-defined glans and a well-marked layer of desquamating cells connects the two parts, prepuce and glans. Sagittal sections of the penis at this stage appear as if an invaginating layer of cells had insinuated itself between the glans and the prepuce, as suggested by Retterer and Neuville.



Text-fig. 6.



Text-fig. 7.

Text-fig. 6. C.S. Glans region of penis from human foetus 100 mm. C.R. length. Note the two sub-glans folds closing off a portion of the desquamating epithelium within the developing glans portion of the urethra.

Text-fig. 7. C.S. Glans region of penis from human foetus 170 mm. C.R. length. The glans urethra is here seen closed off enclosing within it a plug of desquamating epithelium. Note the continuity of the glans tissue with the mesodermal tissue of the prepuce at the frenulum, and the V-shaped line of fusion between the two sides of the prepuce epithelium on its ventral aspect.

These cells in addition form a kind of plug closing off the fossa navicularis and the external opening of the urethra. The cavernous tissue at this stage is well differentiated. The wax-plate reconstruction shows the external opening of the urethra to have advanced to its adult terminal position (text-fig. 5 C).

Coronal sections of the glans region of the penis show in the 100 mm. stage,

that the portion of the urethra which is developed by the closing of the genital folds on the under surface of the penis ends just behind the glans region. Serial sections of this region near the tip of the penis show that the under surface of the glans presents a well-marked groove. This groove is formed by the growing downwards of two narrow but distinct folds of cavernous tissue (text-fig. 6). As the sections were traced in a backward direction these folds were seen to become longer and thicker and presently to fold towards each other in the middle line, meet, and fuse to form that part of the urethra which lies within the glans. In forming this part of the urethral canal the two folds enclose a small mass of the desquamating epithelial cells which forms the thick layer on the whole of the outer surface of the glans. When the folds completely join and the glans portion of the urethra is formed, the desquamating cells thus caught within the canal appeared as a plug of cells as described in the sagittal sections within the fossa navicularis of the 170 mm. C.R. stage (text-fig. 4). The sections at this stage also show the line of fusion of the ventral margins of the overflowing prepuce and a continuity between these skin folds and the sub-glans groove; this continuity forms the frenulum.

The further development of this region is seen in the 170 mm. stage (text-fig. 7) cut in coronal section. Here the urethral canal is found completely formed within the cavernous tissue of the glans. The glans is connected on its ventral side by a fold of tissue to the prepuce. This is the frenulum. The cavernous tissue, as in the earlier stages, appears to be continuous with the connective tissue elements of the prepuce, and the two are not separated from one another by any epithelial or other ectodermal tissue.

DISCUSSION

A study of the sections described shows a series of clearly marked changes in the development of the glans penis and prepuce from a naked terminal glans without a urethral canal to one completely surrounded by a prepuce with an apical urethral opening. At first ectodermal and mesodermal portions of the penis appear to increase in size at approximately the same rate, but from the 40 mm. stage onward, when a slowing in the rate of growth occurs in the condensation of the mesoderm which precedes the differentiation into cavernous tissue, the ectodermal tissues increase greatly in size; and by this inequality of growth they flow over the dorsum of the glans, the lateral margins of the folds becoming less evident as they converge on the ventral surface of the penis to the point of the urethral opening.

The part of the urethral tube which lies within the glans portion of the penis, and which has its long axis disposed in a vertical direction as opposed to the cavernous portion which has its long axis in a transverse plane, and known as the fossa navicularis, is developed as a separate entity from that portion of the urethra which lies within the shaft of the organ. It begins as two folds on the under aspect of the glans, and these meet in the middle line, then by their fusion they close off a canal, the fossa navicularis. The fusion

of these two folds, which are continuous behind with the apical portion of the skin folds of the prepuce, causes the skin folds of this region to be drawn together in the middle line. They meet and fuse there with the result that the loose dorsal folds of prepuce are drawn over the sides down to the under surface of the glans in line with the fusion of the sub-glans folds. The fusion of these two sets of folds gives rise to the frenulum. But if these two folds fail to meet and fuse, the cleft prepuce found in cases of hypospadius of the glans results.

As the closure of the sub-glans folds occurs, they enclose what appears to be a plug of the desquamating epithelial cells. The presence of such a plug of cells at this stage of development has long been recognised, but its origin has been explained in quite a different way. Wood-Jones (5) describes it as a "cell invasion, which burrows into the glans itself, and forms the urethra (at first as a solid plug) within the glans. The urethral plug is broken down by the seventh month of foetal life and the fossa navicularis and lacuna magna are formed."

The sections studied in the present series of observations at no stage showed such a "cell invasion" and from them there appears to be no doubt but that the solid plug is simply a mass of desquamating epithelial cells on the surface of the differentiating glans which have been cut off, trapped as it were, between the margins of the sub-glans folds.

At first the terminal part of the differentiating glans has disposed on its surface a thin layer of desquamating ectodermal cells, which are continuous with those of the so-called epitrichial layer of the general skin. This layer of desquamating cells becomes thicker, and when the skin overflows the glans, it encloses a layer of these cells, giving sections of this part the appearance of an "invaginating layer", as described by Retterer and Neuville.

The breaking down of the cells of this layer was studied by Edington (6), who drew attention to their changing form in the later stages of foetal life. The cells of the glans are columnar in shape with deeply stained nuclei, rapidly merging into the cubical-shaped cells of the epithelium of the prepuce at the frenulum. The layer of cells between the glans and prepuce are large and irregularly polygonal in outline, with faintly stained nuclei, while in places they become flattened, and form "nests" of cells arranged in "whorls". The central cells of these nests degenerate and break down, leaving clefts and cavities, and by the extension of these clefts and their ultimate fusion the preputial space is formed.

A study of the microphotograph shown in Plate I, fig. 1, of a section of the terminal portion of the penis from a foetus of 100 mm. C.R. length shows the continuity of the epitrichial layer of cells on the external surface of the prepuce with the invaginating-like layer of cells between the prepuce and the glans. A "cell-nest" seen in this photograph is shown in higher magnification in Plate I, fig. 2. This shows the "whorled" arrangement of the cells which form the nest, and the degenerative changes in the cell which lies at its central portion. The epitrichial and invaginating-like layer of cell are seen to be

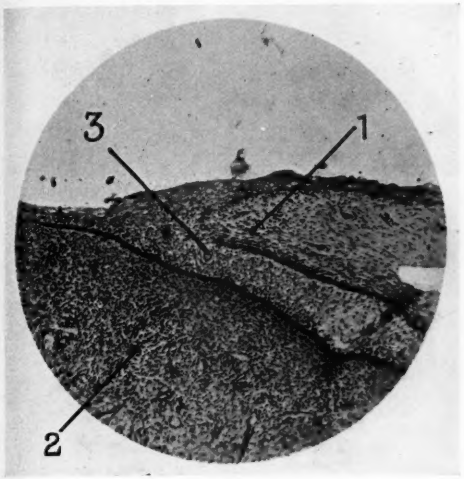


Fig. 1.

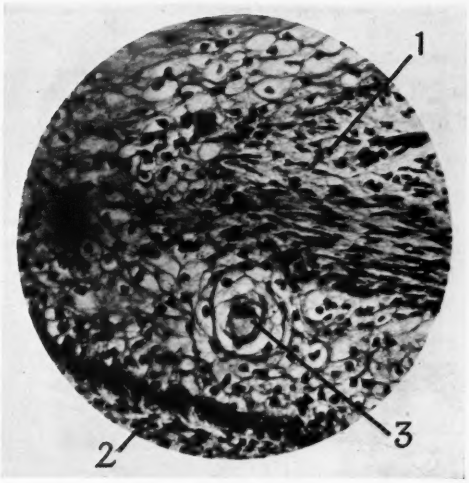


Fig. 2.

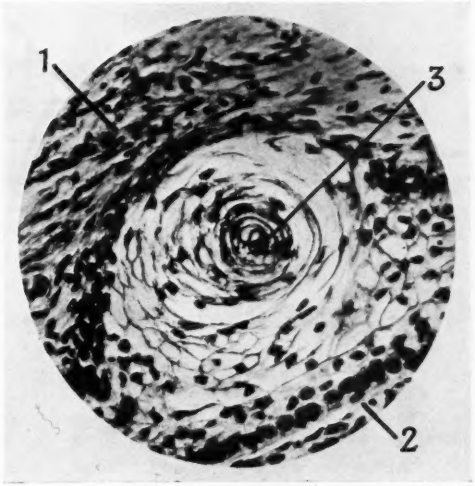


Fig. 3.

composed of the same irregularly flattened polyhedral type. The microphotograph of one of these cell-nests in the invaginating-like layer between the prepuce and glans from a foetus of 170 mm. C.R. length (Plate I, fig. 3) shows a further stage in the degenerative process of its cells, a process which results in the formation of the clefts within this cell layer, which in turn run together to form the definite preputial space.

I should like to acknowledge my indebtedness to Prof. Thomas Walmsley for his kindly criticism and advice in the preparation of this paper.

SUMMARY

1. The glans penis is at first naked and uncovered by a prepuce. It is demarcated from the shaft of the organ by a shallow groove.

2. During differentiation of the cavernous tissue of the penis, growth of this tissue does not occur at so rapid a rate as that of the ectodermal tissues, and the latter becomes thrown into folds on the dorsum of the penis.

3. The folds of ectodermal tissue then appear to flow over the dorsum of the glans as the beginning of the prepuce. The margins of the prepuce are obliquely disposed along the sides of the glans towards the position of the urethral opening, which at this time is placed well back on the ventral surface of the penis.

4. By the further growth of the prepuce its margins flow over the sides of the glans, and are drawn together on its ventral surface by two sub-glans folds, so that the glans is completely surrounded by the prepuce. The line of fusion between the sub-glans folds and the margins of the prepuce form the frenulum.

5. During these changes desquamation of the general ectodermal tissue occurs, and the glans becomes covered by a layer of desquamating cells which is continuous with the epitrichial layer of ectoderm. The prepuce as it flows over the glans enfolds this layer of desquamating cells, and these at a later date degenerate, leaving clefts which in turn run together and form the definitive preputial space.

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EXPLANATION OF PLATE I

Fig. 1. Microphotograph of terminal part of penis from human foetus of 100 mm. C.R. length, to show the continuity of the cells of the epitrichial layer of the prepuce with those of the layer between the prepuce and glans. Pointers: 1, prepuce; 2, glans penis; 3, cell nest.

Fig. 2. Microphotograph of a "cell-nest" in the layer of cells between prepuce and glans, from a human foetus of 100 mm. C.R. length. Pointers as fig. 1.

Fig. 3. Microphotograph of a "cell-nest" in the layer of cell between prepuce and glans, from a human foetus of 170 mm. C.R. length. Pointers as fig. 1.

STUDIES ON THE AREA VASCULOSA OF THE EMBRYO CHICK

I. THE FIRST DIFFERENTIATION OF THE VITELLINE ARTERY

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Sir Halley Stewart Research Fellow,
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I. INTRODUCTION

In 1893, the *Untersuchungen über die Histogenese und Histomechanik des Gefässystems* of R. Thoma was published, which describes the development of the area vasculosa of the chick blastoderm up to the third day of incubation. This was by no means the first time that the origin of blood vessels in the chick blastoderm had been traced, and Virchow (1891) had already described the anatomy of the area vasculosa, which was again studied by Popoff in 1894. Thoma was the first, however, to study the effect of the circulation on the configuration of the vessels of the area vasculosa.

Blood normally begins to circulate at the stage of sixteen somites, i.e. after about thirty-seven hours of incubation, previous to which a more or less uniform network of vessels has differentiated. By the end of the second day of incubation, from those sections of the vessel network through which the flow of blood has been most rapid, larger vessels or arteries have differentiated, in particular from those sections of the network next to the embryonic axis communicating with the primitive aortae.

Experimental proof that the cause of arterial differentiation in the area vasculosa was the stimulus of blood flow was given by Chapman (1918), who destroyed or removed the heart at the sixteen-somite stage from blastoderms in the egg, and continued the incubation of the operated eggs. No differentiation of the arteries of the area vasculosa took place under these conditions.

Similar results were obtained by Clark (1918), who observed the vessels in the transparent fin of the tadpole's tail, and watched the differentiation of particular capillaries into arteries and veins, which were at first in no way morphologically different from surrounding capillaries but which happened preponderatingly to transmit the blood flow in that particular region.

That this conception of arterial differentiation is generally applicable to the development of the intra-embryonic vascular system, was made probable by the results obtained from the study of embryonic material in which the vascular system had been injected. The general conclusion from such studies was that in ontogeny all differentiated blood vessels with the exception of the primitive aortae and aortic arches are preceded by a stage of capillary plexus, from which, it is reasonable to conclude, the various differentiated vessels arise under the influence of the circulation (Mall, 1904; Evans, 1909; Shearer, 1933; Woollard, 1921; Hughes, 1934).

Undifferentiated capillaries arise in ontogeny therefore by self-differentiation, whereas the development of differentiated arteries and veins belongs to the "functional period of development" (Roux, 1881; Huxley and de Beer, 1934).

The first differentiation of arteries does not, however, exhaust the influence of functional adaptation upon the form of the vascular system. As is well known, the angle at which branches are given off from the main artery can be related to the physical laws underlying the fluid friction generated in such systems (Roux, 1879; C. D. Murray, 1926), and indeed, as is shown from evidence in this paper and elsewhere, the form of all vessels is only maintained where blood continues to flow through them.

The present paper is a continuation of the work of Thoma on the area vasculosa of the chick. Thoma describes the histology of the developing area vasculosa only as far as the pre-circulatory differentiation of the vessels. It is the purpose of this paper (*a*) to extend the account of fine structure into the early post-circulatory period, (*b*) to fit into the description of arterial development the part played by the contractility of endothelium (Hammett and Zoll, 1928; Cohn and Lange, 1930), and (*c*) to describe the results obtained from the observation of living blastoderms in which arterial differentiation took place.

II. MATERIAL AND METHODS

The choice of the area vasculosa of the chick blastoderm for a study of the factors concerned with arterial differentiation depends on several circumstances. In the first place, we are following a comparatively rapid series of changes, in that the differentiation of the vitelline artery takes place within the first

twelve hours from the inception of circulation. Secondly, these changes are taking place in a thin sheet of tissue whose vessels are easy to observe in the living state, and which is sufficiently thin to study in the form of whole mounts after histological treatment, which was the method adopted by Thoma. The whole depth of an area vasculosa can be studied with an oil immersion lens of comparatively long working distance, which is an advantage not only in the time saved by avoiding the preparation of serial sections, but in the fact that the shape of nuclei, and the direction of mitotic spindles, are seen with such clearness in relation to their surroundings, a point on which much stress is laid in the present paper. Details of the histological methods used are given in the section following that on vital observation.

Observation of the living area vasculosa

There are two main methods of observing the living chick blastoderm, either in the unbroken yolk, or after explantation of the blastoderm to a non-yolky culture medium *in vitro*. In general, the advantages of the *in ovo* method are that it is less laborious, and that no aseptic precautions are necessary, while on the other hand *in vitro* cultivation makes it possible to perform elaborate operations on the blastoderm and to use transmitted light which is preferable from the photographic standpoint. Among workers on the earliest stages of development, Wetzel (1929), Gräper (1929), and Kopsch (1934), to mention only the most recent, work *in ovo*, while Waddington (1932, and subsequently) employs the tissue-culture technique.

Explantation has been used in the study of the vascular system of the blastoderm by McWhorter and Whipple (1912), by Sabin (1917, 1919) and by Patten and Kramer (1933). These authors have all used hanging drop methods, in that the blastoderms were explanted on to cover-slips with a nutrient medium. By this means, observation under high magnification is possible, but the time of survival is less than when the watch-glass culture vessel is used. The latter is in regular use in this laboratory for explants of large size, and permits of the use of a comparatively large amount of culture medium. Its use for whole blastoderms has been described by Waddington (1932). Such cultures can only be observed with objectives with a working distance of several millimetres.

McWhorter and Whipple used fowl plasma as a culture medium, either pure, or diluted with Locke's solution, and were able to grow blastoderms with three somites to the eighteen-somite stage or with ten-somite embryos for a further thirty-one hours. Sabin used a fluid medium in which her blastoderms survived for periods of five to eight hours, which was the experience of Patten and Kramer, using similar cultural conditions. When, however, the latter authors used larger culture chambers with the same fluid medium, the survival time became from twenty to thirty hours.

Waddington, using a mixture of fowl plasma and embryo extract in watch-glass cultures could grow primitive streak blastoderms for two to three days,

and nine-somite embryos for two days. This is the form of culture vessel which has been employed for the present work, and it has been found that the later the stage at which an embryo is explanted, the less is the total time for which cultivation is possible. The most advanced embryo which I have observed in culture was one of thirty-seven somites with well-marked fore and hind limb-buds, which had been explanted at twelve-somites forty hours previously. It is advantageous when growing embryos in culture to the latest stage possible to include in the medium the "subblastodermic fluid" of P. D. F. Murray (1933), which is the fluid within the vitelline membrane of eggs which have been incubated for three or four days. This when mixed with fowl plasma, and clotted with a trace of embryo extract, forms an admirable medium for the growth of explanted embryos in the second day of incubation, but it has the disadvantage of increasing the tendency of the clot to liquefy. Subblastodermic fluid must form a vehicle for the conveyance to the blastoderm of the white yolk which serves as the food material for the blastoderm in the first three days of incubation, for when centrifuged, a heavy deposit of white yolk results. It is even possible to use subblastodermic fluid alone as a culture medium by floating blastoderms on to discs of filter paper, the edges of which are kept immersed in the fluid. By this means it is possible to charge the culture medium at will.

Where, however, the aim of the investigator is vital observation of the latest stage possible, and where no extensive manipulation of the embryo is desired, the embryos, in the writer's opinion, are best left in the egg. The life of embryos in cultivation when the circulation has been well established is always terminated by an aggregation of the blood corpuscles, which appears to take place quite suddenly. This aggregation presumably does not involve the usual adult clotting reactions, for, according to Pickering and Gladstone (1925), chick blood contains neither fibrinogen nor prothrombin before twelve days of incubation. Embryos in cultivation at thirty somites have always an area vasculosa many times smaller in area than is normal in the egg. Yet multiplication of blood corpuscles seems to proceed at the normal rate, with the result that the vessels of the embryo and area vasculosa are over-full of corpuscles, which causes stagnation of the blood, and subsequent aggregation. Aggregation of corpuscles always seems to follow stagnation, and can be observed normally in the area vasculosa in particular places where arterial differentiation temporarily involves stagnation. Therefore, for the further maintenance in culture of explanted embryos, nutritional considerations do not appear to be the limiting factors.

Observation of embryos in opened eggs with whole yolks is by no means new. It is regularly undertaken in the teaching of chick embryology for a few hours, when the eggs are opened into warm saline, and it was found by Lange (1930) that eggs at the second day of incubation opened into Ringer kept saturated with oxygen when maintained at the proper temperature would continue their development for a further two days. Romanoff (1931) has in-

vestigated thoroughly the conditions for survival in an opened egg. Eggs are opened under sterile conditions, kept covered but well aerated, and maintained at 38° C. and 65 per cent. relative humidity. Under these conditions, when opened during the first day of incubation, all the eggs survive till the third day, half of them till four days, and some to the twentieth day.

I have found that under less carefully controlled conditions, merely by keeping dishes of water on the floor of the incubator, many eggs when opened in Romanoff's manner during the first day of incubation will continue to develop normally for a further four or five days, giving an area vasculosa of normal size. Aseptic precautions do not appear to be necessary. Development of the embryo in the opened egg, as Romanoff points out, permits of an enormous range of vital observation, full advantage of which yet remains to be taken.

For direct observation transmitted light can only be used for the area pellucida of explanted blastoderms; indirect illumination is necessary for the area opaca, and for the whole blastoderm *in ovo*. The "Ultropak" illuminator of Leitz is most convenient as a source of vertical illumination for the blastoderm *in ovo*. The objective and ring condenser system is fitted with a water dipping cone of parallel-sided glass, the lower surface of which is kept in contact with the vitelline membrane, which was kept wet by a slow stream of Ringer. The screw motion which varies the distance between the dipping cone and the objective forms a convenient fine adjustment for the microscope system. The series of photographs (Plate III, figs. 1–5) were taken *in ovo* with this illuminator, using the ×11 objective.

For the observation of the vessels of the area opaca in explanted blastoderms a special method was devised, results obtained with which are shown in Plate I, figs. 1 and 2. The vessels were injected by means of a micro-manipulator with an Indian ink sufficiently non-toxic not to interfere with the usual life of the blastoderm in culture. This Indian ink was obtained in the first place by rubbing up the ordinary commercial stick of solid Indian ink in a phosphate buffer, and dialysing away toxic substances in a collodion membrane. This buffered solution of colloidal carbon withstood autoclaving, and could be made into a physiological saline by adding the necessary salts immediately before use. Later it was found that a stick of solid Indian ink free of the noxious substances of the commercial article could be made by mixing finely divided carbon in the form known as "gas black" with a strong solution of gelatin, and then desiccating the mixture.

This vital injection technique suffered from one main disadvantage, namely, that after injection a severe contraction of the injected vessels frequently resulted. Vascular endothelium, it is now recognised, is a contractile tissue, and a subsequent section is devoted to a consideration of this phenomenon. Moreover, since it was found that the state of expansion of vessels is of importance with regard to their differentiation into arteries, it was inadvisable solely to rely on vital injection to give a normal picture of the differentiation of

an artery. We shall see that the same objection applies even to the explantation of blastoderms in this connection.

Furthermore, since a mere description of the gross anatomical changes which take place in the differentiation of an artery loses much of its value if these changes are not correlated with the cell processes involved, attention is almost exclusively directed in the present paper to arterial differentiation in that region where the fine structure can be most readily and completely studied. This region is the area pellucida, where arterial differentiation first occurs, and where whole mounts treated in the manner described below can be examined with an oil immersion objective.

Histological methods

The suitability of the area pellucida for observation in whole mounts depends on the use of the Feulgen reagent for nucleic acid, which not only stains nuclei, but yolk in addition. Thoma's own preparations were stained in alum carmine, which allows the mesoderm of the area opaca to be observed to some extent, since the yolk is not stained so deeply as with the Feulgen reagent. In the area pellucida, however, where little or no yolk is present in the endoderm, nuclei stand out much more clearly in Feulgen preparations than in those stained in alum carmine, which is of great advantage where many layers of cells are to be observed, as in stages where arterial differentiation has taken place. Thoma's observations under high power leave off before this stage has been reached.

The blastoderms were fixed in Zenker, and washed in distilled water. For stages in which a fully developed exocoelom is present, the ectoderm and outer coelomic wall were dissected off when in water; this dissection near the embryonic axis involved the removal of large masses of mesenchyme dorsal to the coelom in the neighbourhood of the posterior somites, revealing the vessels below. By this means, the total depth of the preparations was reduced, and penetration by the various reagents employed was facilitated.

The preparation and use of the Feulgen reagent is described in Romeis (1932). Preparatory to its use, the material is hydrolysed in normal hydrochloric acid at 50° C., for which process various times are suggested by Romeis for different objects. I have found that for my material forty minutes' hydrolysis gave satisfactory results. Yolk stains in Feulgen without hydrolysis, whereas as the hydrolysis proceeds the nuclear material is rendered capable of taking a deeper stain. The staining of substances other than nucleic acid by the Feulgen reagent has been noticed by other workers. After hydrolysis, the blastoderms are washed in distilled water, and left over-night in the Feulgen. They are then washed in three changes of SO_2 water, as described by Romeis, dehydrated, and counter-stained in light green dissolved in absolute alcohol. They are then brought into 1 per cent. collodion dissolved in methyl benzoate, which is the clearing technique elaborated by Peterfi, and described in Romeis. The blastoderms are mounted in a second change of this medium, and the preparations

ringed round with paraffin wax with which methyl benzoate does not mix, when they form almost permanent preparations. Some last indefinitely, but others require the addition of more methyl benzoate at intervals. The advantage of this medium is that sectioning of the material is possible at any time subsequently, since no hardening takes place.

When an already well-stained blastoderm is to be sectioned it is brought into benzene for a few minutes, and then embedded in wax in the ordinary way. Sections are cut at 6μ and when mounted need only to be re-counter-stained in light green in absolute alcohol.

When studying whole mounts with an oil immersion lens, a less viscous immersion medium than the usual immersion oil must be used, and for this pure methyl benzoate is satisfactory.

A final point regarding the methods employed in this work is one concerning the relationship between the time of incubation of eggs, and the stage of development of the blastoderm. It is well known that in a group of eggs incubated for the same time, there may be considerable divergences in the degree of development of the embryos; these divergences are more marked in the first two days of incubation, and in eggs incubated during the winter months. In my experience, they are related to a variation in the period after the commencement of incubation before development is resumed, for once growth begins in the incubator it proceeds at the normal rate. Thus, in the winter especially, the phrase "embryo of twenty hours' incubation" has very little meaning.

Consequently, we are left with the number of somites as the only convenient measure of development. To reduce the number of somites to a standard time scale, the data in Lillie's *Embryology of the Chick* were plotted, and the resulting curve gives a measure of the time required for the addition to a living blastoderm of a certain number of somites, which holds true for blastoderms *in ovo*, and those *in vitro* when explanted after the ten-somite stage. Waddington (1932) describes the extent to which development *in vitro* is slower than *in ovo* when earlier stages are explanted. In estimating the number of somites in embryos in which there are at least twenty-four somites, their number posterior to the vitelline artery is counted, for this artery from this stage onwards is always opposite the twenty-third somite.

Even the number of somites is not a fully satisfactory criterion of the stage of development, for where the number of somites present is much less than that corresponding to the period of incubation in Lillie's table, the stage to which other structures, such as the blood vessels, are developed is ahead of that corresponding to the number of somites actually present.

III. THE PRE-CIRCULATORY DEVELOPMENT OF THE AREA VASCULOSA

As stated in the Introduction, blood normally begins to circulate in the chick blastoderm at the stage of sixteen-somites. By this time, a network of freely communicating blood vessels in the area vasculosa has developed, the

form of which is of the greatest importance in regard to the paths along which circulation will first take place, and for the ultimate differentiation of arteries within the vessel network.

A full discussion on the histogenesis of the extra-embryonic endothelium is postponed for a later communication. The main accounts of this process in the literature do not fully accord with one another (Thoma, 1893; Rückert and Mollier, 1906; Sabin, 1917, 1919). Modern workers, however, agree that endothelium is exclusively derived from the mesoderm, and originates *in loco* (McClure, 1921), contrary to the original angioblast theory of His (1900). There is further agreement, moreover, that the first vessel primordia take the form of isolated vascular spaces. They increase in size, and the walls of adjacent spaces become apposed, and then are resorbed at the point of contact.

The development of the vessel network

The first stage in vessel formation is the differentiation of isolated vascular spaces. Coalescence of adjacent spaces leads to the formation of a continuous vessel of comparatively large diameter, but where the first vessel spaces are far apart, they are joined together by means of the outgrowths known as "sprouts", the cells of which are derived by proliferation from the wall of an already formed endothelial space; the lumen of the sprout is formed largely by extension of the parent lumen, which may join up with small independent cavities which appear within the sprout. Vessels formed in this manner have at first narrow lumina, and in any part of the area vasculosa the general size of lumen depends on the relative proportion in which primary vessel formation and growth through sprouting have been concerned with the formation of the vessel network. In general, the elongation of vessels, or of vessel primordia, causes the vessel lumina to be of small diameter. These considerations are of great importance in relation to the paths in the vessel network along which blood will flow when the circulation begins, for the flow will be largely restricted to the largest vessels, which will offer least resistance.

Vessel differentiation proceeds antero-posteriorly, and from the area opaca to the pellucida. It is this latter circumstance, coupled with the fact that sprout formation is most marked in the anterior and middle regions of the pellucida, which led the early workers on the subject to formulate the doctrine of the vascularisation of the embryo from without.

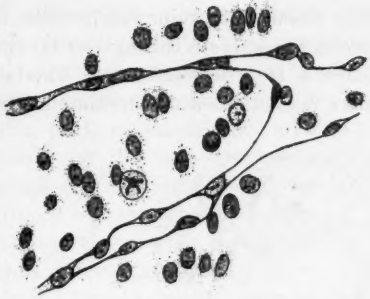
Isolated vessel primordia first appear in the area pellucida between the stages of three to six somites, first at the pellucida margin, and then nearer the embryo. Their first appearance and subsequent combination was most beautifully recorded in living material by McWhorter and Whipple in a series of photographs taken at a magnification of $\times 500$. My own photographs of a living blastoderm cultivated *in vitro* (Plate II, figs. 1–5) are taken at the much lower magnification of $\times 34$, and cover a period of about fourteen hours. From comparison with fixed material, it is apparent that the stage in the photographs at which the outlines of vessels are first distinctly seen, corresponds

with that of the full differentiation of the network, with fully communicating lumina. This stage is first reached in the region of the pellucida opposite the somites when their number is between ten and eleven (Plate II, figs. 2 and 3), when the network is made up of vessels of small calibre. Then, immediately behind the somites there differentiates an area of vessels of larger calibre, which unlike those more anteriorly which have already differentiated, communicate with the dorsal aortae on each side. These are the vessels from which the proximal sections of the vitelline arteries will ultimately arise. Their communication with the aortae is best shown in injected preparations, such as those figured by Popoff (1894), but they may be readily recognised at each stage by their large lumina. If they are followed from their first appearance onwards, it is apparent that they continue to occupy a position just behind the last somite, despite the fact that the number of somites is continually being added to from behind. This must mean that as the somites form they move forward as a whole, and that the anterior end of the embryo moves forward relative to the blastoderm. Now the heart connects with the vessels of the area pellucida through the sinus, and since from the ten-somite stage onwards the level of the sinus is in the neighbourhood of the first somite, this must mean that the network of vessels in the area pellucida elongates as a whole in the direction of the embryonic axis, though not to the same extent as the axis, for at seven somites the heart is well in front of the first somite, while at the twenty-somite stage the first somite occupies the same transverse level as the ventricle. But allowing both for this and for the longitudinal crowding of the somites which takes place during development, some elongation of the vessels of the area pellucida opposite the somites does take place, and is reflected in the details of their arrangement. The forward movement of the somites relative to the blastoderm can be seen in Plate II, figs 1–5, by comparing the position of the first somite relative to the yolk granule (*g*) lying on the surface of the ectoderm of the area pellucida.

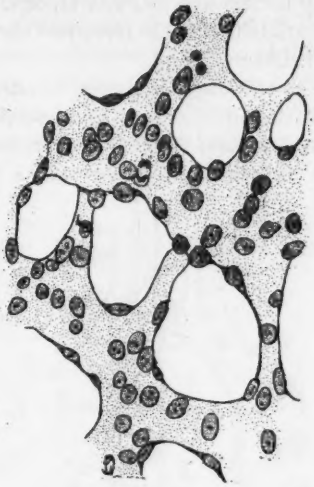
Plate II, fig. 6 is a photograph of a blastoderm at the eleven-somite stage, in which the three areas (*a*), (*b*) and (*c*) are again shown as high-power drawings in text-figs. 1, 2 and 3. Opposite the seventh somite (text-fig. 2) those components of the vessel network at right angles to the embryonic axis are of larger calibre than those parallel to the axis, and in the latter adjacent lumina are not yet fully patent, in that some resorption of adjacent endothelial walls has yet to take place. These narrower components of the vessel network are the late stages of "sprouts" from the larger, already differentiated transverse vessels, and these sprouts elongate with the general extension of the area pellucida vessels in this region.

The vessels opposite the seventh somite contrast strongly with the vessels of larger calibre of the future vitelline artery area behind the somites (text-fig. 3); posterior to this area, the vessels at this stage consist of unjoined vascular spaces surrounding blood islands.

The differentiation of the "vitelline artery vessels", as these may be termed,



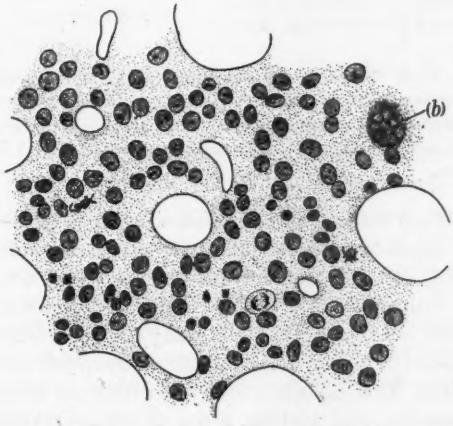
Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Drawing of the area (*a*) of the eleven-somite blastoderm shown in Plate II, fig. 6. Area (*a*) is in the pellucida opposite the first somite. The drawing is largely in the plane of the vessel lumina. ×360.

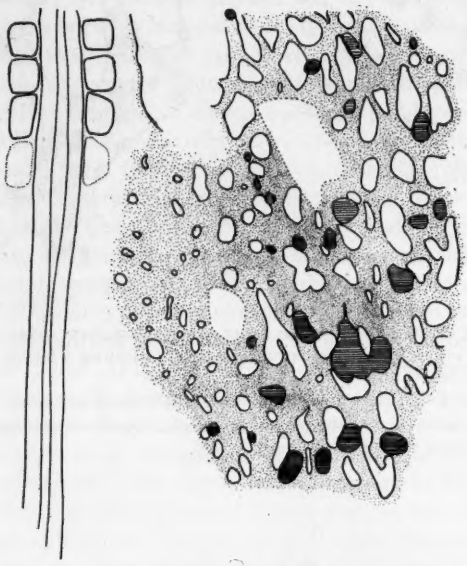
Text-fig. 2. Drawing of the area (*a*) of the eleven-somite blastoderm shown in Plate II, fig. 6. Area (*b*) is in the pellucida opposite the seventh somite. The drawing is largely in the plane of the upper endothelial wall of the network. ×400.



Text-fig. 3. Drawing of the area (*c*) of the eleven-somite blastoderm shown in Plate II, fig. 6. Area (*c*) is in the vitelline artery area in the pellucida, behind the somites. The drawing is in the plane of the upper wall of the vessels, except for the blood island within the lumen at (*b*). ×400.

may be followed in Plate II, figs. 3 and 4, in which it appears that they differentiate first at the margin of the pellucida, and are of large calibre from their first differentiation.

At the stage of sixteen somites, when blood first begins to circulate, differentiation of vessels from isolated spaces is complete throughout the area vasculosa, and vessel lumina are everywhere in free communication. Caudal to the "vitelline artery vessels" a vascular network has differentiated whose



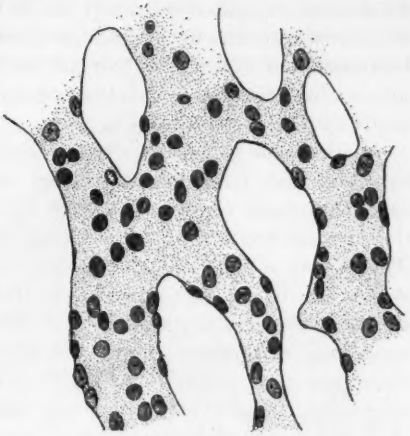
Text-fig. 4. Drawing of the vitelline artery area of a sixteen-somite blastoderm taken from the egg. The blood islands are cross-hatched, and the depth of shading of the vessels is intended to represent the distance between the upper and lower endothelial walls of the vessel network, according to the key in text-fig. 15. The areas enclosed by dotted lines are gaps in the preparation. ×52.

vessels are of medium diameter. The vitelline artery vessels have the largest lumina, and the smallest intervascular spaces in the whole area vasculosa, whereas that region of the vessel network anterior to the vitelline artery area and nearest the embryonic axis has the smallest lumina and largest intervascular spaces, the intervascular spaces being elongated still further with the subsequent extension of this area as a whole, consequent on the formation of the last five somites. Text-fig. 4 is a low-power drawing of the vitelline artery area at sixteen somites, and text-fig. 5 one at higher magnification showing elongated vessels of the same blastoderm opposite the seventh somite.

Thus, when the circulation first takes place, blood flows out from the aortae, through the vitelline artery vessels, and takes a wide path through the

area vasculosa before returning to the heart. Blood issuing from the aortae does not take the shortest path back to the heart through the vessels nearest the embryonic axis, because of the resistance offered to the flow by reason of their small calibre. Less resistance being offered by a longer path through larger vessels farther out in the area vasculosa, the circulation takes place through distal rather than proximal vessels, and in consequence affects as big an area of the whole vessel network as possible.

The path taken in the area vasculosa by the first circulating blood was described by Thoma, who saw that it is determined by factors other than those which cause the ultimate differentiation of an artery within the vessel network. Arterial differentiation is due to the effects of long-continued blood flow, but the operation of the afore-mentioned factors is necessarily antecedent to the later differentiation which is the result of circulation, in that they determine the path along which blood shall flow at a time before circulation begins. The differentiation of arteries has therefore two aspects, one of self-differentiation before the circulation takes place, and the other of functional differentiation in response to the circulation.



Text-fig. 5. Drawing of a small area in the vessel network of the area pellucida, opposite the seventh somite, of the sixteen-somite blastoderm *in ovo* of text-fig. 10. The drawing is in the plane of the upper wall of the network. ×360.

That this is true in a wider sense also, was pointed out by Thoma, namely in relation to the relative times in development at which the heart begins to beat effectively, and at which a fully formed vessel network is differentiated. We know that in the chick and elsewhere, the latter event precedes the former. Were this not so, blood would flow exclusively through those channels which were sufficiently differentiated to transmit a circulation, and which would thereupon begin to transform themselves by enlargement into arteries, while vessels of smaller calibre subsequently differentiated would offer too high a resistance ever to transmit blood under these conditions. It is essential for the ultimate development of a vascular system consisting of arteries, capillaries, and veins, that there should be presented to the first flow of blood a more or less uniform network of small vessels through which blood will not flow exclusively along one particular path.

Embryonic endothelium as a contractile tissue

That vascular endothelium is of itself contractile was first demonstrated by Clark and Clark (1925) who showed that in the capillaries of the transparent fin of the tail of the frog tadpole, contraction can take place prior to the development of the adventitial Rouget cells, thus denying the general applicability of the conception of Vimtrup (1922) that the contractility of fully differentiated capillaries is solely due to these particular cells. The capillaries of the area vasculosa in the chick are devoid of Rouget cells, and were shown by Hammett and Zoll (1928) to react to the application of carbon dioxide in solution by a vigorous contraction, a response which was not evoked by changes in *p*H within the physiological range.

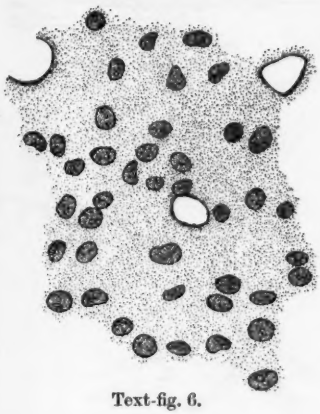
Work on the physiology of the vessels of the area vasculosa was continued by Cohn and Lange (1930). They showed that no innervation of the extra-embryonic vessels took place throughout the incubation period, that these vessels would react to mechanical and electrical stimuli, and to chemical stimuli such as ammonia. They further showed that the irritability of these vessels was in inverse proportion to their calibre, and that in general, weak stimulation caused dilation of vessels, which was followed by contraction on the application of stronger stimuli. However, in their experiments on the precirculatory area vasculosa "at the time when there are only blood islands and no regular flow of blood, contraction and dilation do not occur".

In the course of the present work, contraction of area vasculosa vessels was first encountered in the use of the technique of vital injection described in the above section of methods. Some fifteen minutes after the injection of the dialysed Indian ink, the vessels in that section of area vasculosa which had received the ink least diluted by the blood gradually contracted until their lumina would no longer transmit fluid. Recovery generally took place during the next one or two hours. This could occur equally before or after the first appearance of the circulation.

Cultivation of whole blastoderms or of fragments of area vasculosa without injection and under the conditions of tissue culture employed in this work leads in general to a dilation of the vessels, which persists and is not followed by a contraction to the normal state unless circulation of blood through them takes place. This fact has had considerable influence on the progress of this work, for since the state of expansion of vessels is of the greatest importance for their differentiation into arteries, it has not been possible to rely exclusively on the use of explanted blastoderms for the observation of arterial differentiation.

Dilation of extra-embryonic vessels is shown equally in explanted blastoderms in which the whole embryo or the heart has been removed, or in those in which the heart although beating, fails to propel blood through the vessels. Failure of the heart beat to cause circulation in blastoderms grown *in vitro* from the earliest stages has been noticed by Waddington (1932). Text-fig. 6 is a drawing of a small section of the area pellucida of a blastoderm in which the embryo was

removed at the stage of sixteen somites, and the rest of the blastoderm cultivated *in vitro* for a period of 27 hours, and subsequently mounted whole, as described in the section on histological methods. The dilation appears in surface view as a decrease in the area of the intervascular spaces, and on focusing from the upper surface of the vessel network through the lumen to the lower surface in contact with the endoderm, it appears from comparison with undilated vessels, that dilation has also increased the dimension of the vessel network at right angles to the plane of the blastoderm. This increase in the area of the endothelium does not involve any increase in the number of cells present, and throughout the endothelium mitotic figures are exceedingly rare.



Text-fig. 6.

Text-fig. 7.

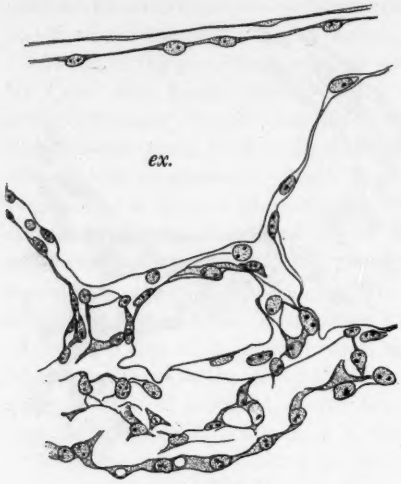
Text-fig. 6. Dilated vessels of a small area of a blastoderm cultivated *in vitro* without the embryo for twenty-seven hours. The upper wall of the vessel network is shown. ×400.

Text-fig. 7. Vessels of a small area of a blastoderm cultivated under the same conditions as that of text-fig. 1, but in which the injection of Indian ink caused contraction. The ink has precipitated on the inner walls of the vessels, and the endothelial nuclei have elongated. ×400.

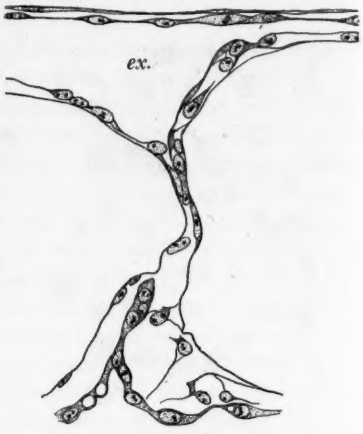
Moreover, these dilated vessels can be made to contract equally with those of blastoderms which have not undergone this process of dilation through cultivation *in vitro*. Plate III, fig. 6 is a photograph of a blastoderm cultivated without the embryo under the same conditions and for the same time as that of text-fig. 6, but in which thirty minutes before fixation an injection into the vessels of the Indian ink used in the vital injection experiments had been made. Contraction of the heavily dilated vessels began a few minutes after injection, and gradually became more severe. The ink precipitated on the walls of the vessels, and shows the extent of the contraction, and the corresponding increase in the size of the intervascular spaces. A drawing of this blastoderm under high power is seen in text-fig. 7, which shows the effect of this contraction on

the shape of the endothelial nuclei, a matter which is discussed at length below.

Dilation of the vessels of explanted blastoderms in which no circulation takes place finally results in the obliteration of the intervascular spaces, which gradually become smaller until the bordering endothelial wall becomes a solid vertical strand. The stages in this process, as seen in transverse section, are illustrated in text-figs. 8–11, which are drawn from a section through a blastoderm cultivated *in vitro* in which circulation failed to take place at the appropriate time, and in which, in consequence, dilation of all vessels took place. Dilation in this blastoderm happened to be least anteriorly, and progressively



Text-fig. 8.



Text-fig. 9.

Text-fig. 8. A small area of a section through the area pellucida of a blastoderm cultivated *in vitro*, showing three adjacent unfused vessels. *ex.* exocoelom. ×400.

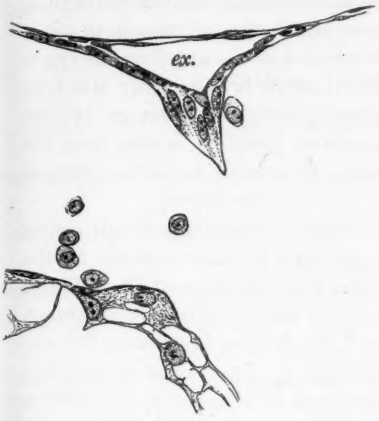
Text-fig. 9. Another part of the same section as that of text-fig. 3, showing two adjacent vessels, the intervascular spaces between being now an intervascular strand. *ex.* exocoelom. ×400.

more severe in the caudal direction. Of these figures, text-fig. 8 is the most cranial in the section, and the others represent successive stages in the process passing caudally. After the apposition of the endothelia from opposite sides of the intervascular space as seen in text-fig. 9, a stage which I call that of the "intervascular strand", the strand is resorbed as seen in text-fig. 10, where the dorsal end of the strand still hangs from the upper endothelial wall.

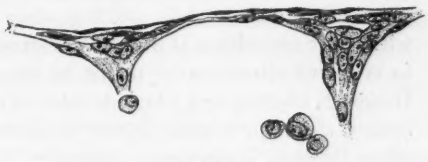
Meanwhile dilation has also been taking place in the dorso-ventral direction at the expense of the extra-embryonic coelom, which is gradually obliterated during the process, until in text-fig. 11, where the lower endothelial wall is now too far from the upper to be drawn in the same figure, ectoderm, somatopleure, splanchnopleure, and endothelium are all in contact, and small isolated spaces are all that remain of the extra-embryonic coelom. The same process takes

place wherever vascular spaces fuse. Within the differentiating artery, intervascular spaces are ultimately represented by intervascular strands (text-fig. 12).

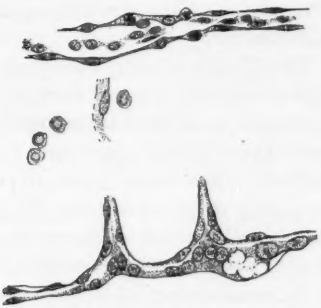
It is not clear what factor in the conditions of tissue culture is responsible for the dilation of these vessels where no circulation takes place. Dilation of vessels under these conditions has been noticed previously by Strangeways and



Text-fig. 10.



Text-fig. 11.



Text-fig. 12.

Text-fig. 10. Another part of the same section as that of text-figs. 3 and 4, in which the intervascular strand between two adjacent vessels has broken. The exocoelom (*ex.*) becomes smaller as the vessels continually dilate. ×400.

Text-fig. 11. Another part of the same section as that of text-figs. 3, 4 and 5. This shows the end stage of the process of dilation and fusion of vessels, in which the exocoelom is almost entirely obliterated. ×400.

Text-fig. 12. Section through the vitelline artery of a twenty-two-somite blastoderm cultivated *in vitro*, in which circulation took place for eight and a half hours. In the floor of the differentiated artery are shown two severed intervascular strands. ×400.

Fell (1926) in their early work on the growth of chick limb-buds in tube cultures. This dilation affected spaces within the explants other than the lumina of vessels, such as cavities between ectoderm and mesoderm and within areas of necrosis within the mesoderm, and by the coalescence of these different cavities large cysts were formed. Cyst formation was most marked where media of a more fluid consistency were employed.

When blastoderms are explanted in the pre-circulatory period and successfully circulate at the appropriate time, after the circulation has been in progress for some hours a general contraction of all vessels takes place, after which the degree of dilation of the vessels corresponds with those of blastoderms at the

same stage *in ovo*. This general contraction only takes place *in vitro* where the circulation has been established, for otherwise progressive dilation results. Where circulation is prevented in blastoderms *in ovo*, as in the experiments of Chapman, all extra-embryonic vessels finally contract.

Since it was shown by Hammett and Zoll that carbon dioxide causes extreme contraction of the vessels, it might be possible that the normal carbon dioxide tension within the egg was responsible for maintaining a state of tonic contraction of the vessels, and that since explanted blastoderms are in contact with a free air surface at which the carbon dioxide tension might be lower than *in ovo*, this circumstance might be the cause of dilation of vessels *in vitro*. However, blastoderms which develop *in ovo* exposed to the air when the egg is opened show the normal degree of contraction, which is apparently also true when the yolk is decanted into warm Ringer through which oxygen is kept bubbling (Lange, 1930). Moreover, the contraction resulting *in vitro* from the development of the circulation cannot readily be related to carbon dioxide concentration.

The dilation of extra-embryonic vessels under the conditions of cultivation employed in this present work is probably part of a general tendency for the accumulation of fluid in intercellular spaces. The thickness of the layer of mesenchyme which forms above the differentiated vitelline artery is very much greater in blastoderms grown *in vitro* (Plate I, fig. 3) than in those at the corresponding stage taken from the egg (Plate I, fig. 4). This is due in part to a greater proliferation of mesenchyme *in vitro*, but even more to the greater distance between the mesenchymal nuclei, whose cells connect with each other by the anastomosis of long slender processes forming a meshwork with very large spaces in which fluid accumulates.

It remains to enquire whether anything corresponding to the dilation of vessels of blastoderms cultivated *in vitro* takes place normally *in ovo*. There can be no general tendency for the progressive dilation of vessels *in ovo*, where vessels through which no circulation ever takes place ultimately contract, as is seen in the experiments of Chapman (1918). But when the vessel network is first fully differentiated, the vessels tend to dilate until blood begins to circulate, as can be seen from Table I, and also from comparison of the text-figures illustrating particular regions of the area pellucida network at its first differentiation with similar figures of the same regions at the sixteen-somite stage. Thus in the vitelline artery area, although the intervascular spaces are small at eleven somites (text-fig. 3), they are still smaller at sixteen somites (text-fig. 4).

IV. THE EARLY POST-CIRCULATORY DIFFERENTIATION OF THE AREA VASCULOSA

In this paper the description of the changes in the area vasculosa which follow the development of the circulation is limited to those changes seen in the area pellucida in the neighbourhood of the "vitelline artery vessels" whose

first differentiation is described in the preceding section. These post-circulatory changes appear first in these vessels, and subsequently similar changes take place elsewhere in the area vasculosa along the path of differentiating arteries. Description is limited to this region, because the absence of yolk in the underlying endoderm facilitates observation both in the living and in the fixed condition. The differentiation of the vitelline artery is described first for blastoderms cultivated *in vitro*, and then for blastoderms *in ovo*. Certain differences are encountered between arterial differentiation under these two conditions, due to the tendency for vessels to dilate *in vitro*, a tendency which is one aspect of the contractility of endothelium.

Table I. *Vessels of the area pellucida.*

No. of somites	Vessels opposite middle somites	Amount of mitosis	Vitelline artery vessels	Amount of mitosis	Vessels in posterior region	Amount of mitosis
			Pre-circulatory *in ovo*			
11 (Text-figs. 7, 8, 9)	Not dilated	Few	Dilation moderate	Frequent	Unjoined Anlagen	Frequent
13	Dilation small	Few	Widely dilated	Some	Differentiated, not dilated	Few
16 (text-figs. 10, 12)	Dilation small	Few	Widely dilated	Some	Dilation small	Few
			Post-circulatory *in ovo*			
24 (text-fig. 19)	Dilation very small	Very few	Narrow irregular artery	Few	Dilation very small	Few
26 (text-figs. 20, 24)	Dilation very small	Some	Regular artery	Frequent	Dilation very small	Some
			Post-circulatory *in vitro*			
19, 4 hours' circulation (text-fig. 13)	Dilation moderate	Very few	Fully dilated	Few	Dilation moderate	Few
20, 6 hours' circulation (text-fig. 15)	Dilation small	Very few	Fully dilated	Some	Dilation moderate	Few
27, 9 hours' circulation (text-fig. 16)	Dilation small	Very few	Wide artery	Some	Dilation moderate	Few
30, 13 hours' circulation	Dilation small	Very few	Wide artery	Some	Dilation small	Few
			In vitro after 16 somites without circulation			
22, 7 hours after 16 somites (text-fig. 28)	Dilation moderate	Very few	Fully dilated	Very few	Dilation moderate	Very few

Arterial differentiation in blastoderms cultivated in vitro

In studying the effects of the circulation in an explanted blastoderm where the heart is beating effectively, it is first necessary to determine the part played by that tendency for the vessels to dilate which is always shown in blastoderms *in vitro*, but which is counteracted when circulation takes place.

Plate II, figs. 1–5 are from a series of photographs of a blastoderm cultivated *in vitro* in which no circulation took place. Circulation normally begins at the stage of sixteen somites, and, therefore, this series represents normal development up to this stage (Plate II, figs. 1–4). Plate II, fig. 5, shows the twenty-somite stage, four and a half hours after circulation would normally have taken place. It will be noticed that dilation, as viewed in superficial aspect, is everywhere complete, except in the vessels opposite the region extending from the twelfth to the nineteenth somite, in the area where elongation takes place, which, as explained in the section above, has the effect of restricting the size of the lumina *in ovo*. When no circulation takes place *in*

vitro, these vessels are the last to dilate. In another series of experiments where the heart was removed from a series of blastoderms before explantation, six and a half hours after the period when circulation would normally have taken place, dilation of vessels was everywhere complete except for two regions of the vessel network. The vessels which differentiate subsequent to the appearance of the circulation caudal to the vitelline artery area were not dilated, and those opposite the middle group of somites were only moderately dilated, much as in Plate II, fig. 5.

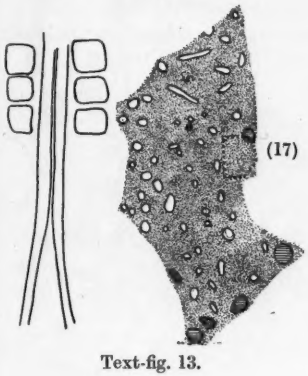
We are now in a position to compare the vessels of blastoderms cultivated *in vitro* where no circulation takes place with those in which blood begins to flow at the appropriate time, and in which a normal flow is maintained for known periods. Text-figs. 13 and 14 represent the vessels of the vitelline artery area of blastoderms which were fixed after circulation had been in progress for four and six hours respectively. Only the vitelline artery area is drawn, for the vessels anterior thereto are covered by a layer of mesenchyme so thick that accurate representation is impossible. It is possible to see, however, that whereas the vessels of the vitelline artery area are widely dilated, the vessels anterior to this area are of small diameter, and passing cranially, as soon as the connection with the aorta is lost, there is an abrupt transition to undilated vessels. Similarly, posterior to the vitelline artery area, and indeed generally throughout the area vasculosa, the dilation of vessels is less extreme when circulation has taken place. The first effect of the circulation, therefore, is to counteract in some measure the dilating influence of the conditions of cultivation. This influence, moreover, is progressive, for after six hours of circulation, the vessels of the area vasculosa generally are less dilated than those through which flow has been in progress for four hours.

Although in the blastoderms of text-figs. 13 and 14 the vitelline artery vessels anteriorly pass abruptly into very much smaller vessels, there is as yet free communication of lumina between these two regions of the area vasculosa. The loss of this free communication of lumina between the vitelline artery area and the vessels to either side leads ultimately to the differentiation of the vitelline artery itself. This process is complete after nine hours of circulation, and the appearance of the vitelline artery area at this stage is shown in text-fig. 16. The embryo in this blastoderm has twenty-seven somites, and circulation did not begin until the stage of nineteen somites. Normally, the circulation would have begun at sixteen somites, and there would be twenty-three somites by the time the circulation had been in progress for nine hours. By comparing blastoderms of slightly different stages in which, however, circulation has been in progress for the same time, it is seen that the time during which blood has flowed, rather than the age of the embryo, is the determining factor in arterial differentiation.

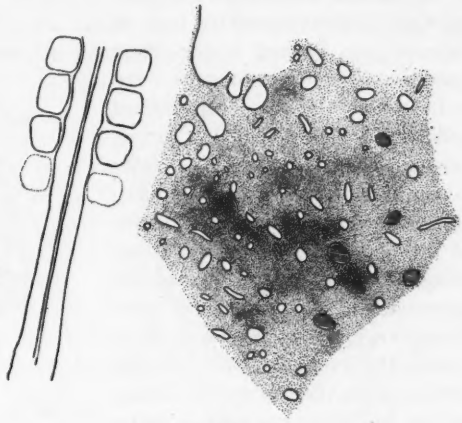
After nine hours' circulation, besides the isolation of the vitelline artery, there has been a general contraction of all other vessels throughout the area vasculosa. Only within the artery, which proximally has been formed from a region of the vessel network which is at least two network meshes wide, are

small intervascular spaces still to be found. Since the vessels adjacent to the vitelline artery area were somewhat contracted before this period, differentiation of the artery on its anterior border has meant only its isolation from neighbouring vessels. On the posterior border, however, neighbouring vessels have both contracted in diameter, and have undergone isolation.

The small intervascular spaces within the artery are all in process of forming intervascular strands, and ultimately disappear in exactly the same way in which neighbouring vessels fuse *in vitro* when no circulation takes place, as



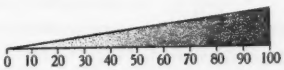
Text-fig. 13.



Text-fig. 14.

Text-fig. 13. Drawing of the vitelline artery area of a twenty-somite blastoderm cultivated *in vitro*, in which circulation took place for four hours. The area (17) is drawn at a higher magnification in text-fig. 17. × 52.

Text-fig. 14. Drawing of the vitelline artery area of a twenty-one-somite blastoderm cultivated *in vitro* in which circulation took place for six hours. × 52.



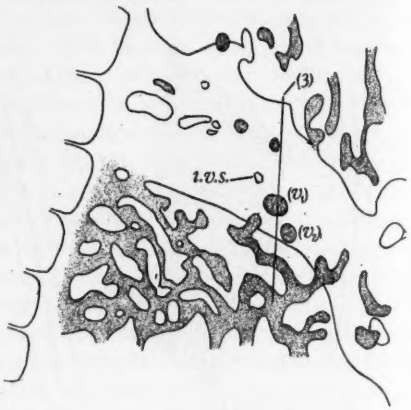
Text-fig. 15. Key to the depth of shading of the vessel network of text-figs. 4, 13, 14 and 20. The numbers represent distance in (μ) between the upper and lower endothelial walls. × 52.

described above. In text-fig. 16, the intervascular space in which this process is farthest advanced is that marked (*i.v.s.*), which is now a strand, from which a long ridge leads in either direction along the artery, as shown in the section of which Plate I, fig. 3 is a photograph. Text-fig. 12 is a section through the differentiated vitelline artery of an explanted blastoderm in which circulation was maintained *in vitro* for eight and a half hours, and shows two severed intervascular strands whose ends are attached to the lower wall of the vessel.

The vessels to either side of the vitelline artery with which they have now lost their connection will form part of a plexus of vessels which is venous in

function, and will return blood from the caudal region of the area vasculosa, passing dorsal to the vitelline artery. The formation of this dorsal venous plexus in blastoderms cultivated *in vitro* is intimately related to the profuse production of mesenchyme under these conditions. For the dorsal venous plexus over the vitelline artery is formed not only by the growth of the severed vessels over the artery, but also by a linking up with small independent vascular spaces which have differentiated within the thick layer of mesenchyme dorsal to the artery.

It was mentioned above in passing that the thickness of the layer of mesenchyme formed under these conditions is due to two causes, viz. to the presence of a larger number of cells than in blastoderms at a corresponding stage *in ovo*, and to a greater accumulation of intercellular fluid, this being the more important factor of the two. The cell bodies within this mesenchyme give off long slender processes, which anastomose with those of neighbouring cells. In comparing the amount of mesenchyme present with the degree of dilation of the neighbouring vessels under different conditions, it is apparent that when dilated vessels contract fluid passes through the vessel wall from the lumen to the surrounding mesenchyme, and that in general dilation of vessels and volume of the mesenchyme are in inverse proportion to each other. In blastoderms which have been cultivated without circulation after the sixteen-somite stage, and in which all the vessels are dilated, mesenchyme cells adjacent to the vessels are in close contact with the endothelium, from which they are not sharply demarcated by the long anastomosing processes which the cells possess when there is much intercellular fluid. Under these conditions the mesenchyme is similar to that of blastoderms *in ovo*, as seen in the neighbourhood of the differentiated vitelline artery in Plate I, fig. 4.

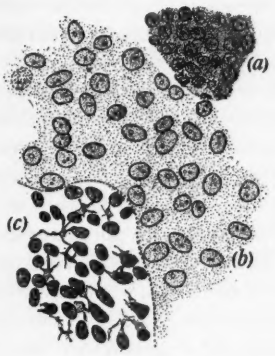


Text-fig. 16. Reconstruction of the vitelline artery area of a blastoderm of twenty-seven somites which was cultivated *in vitro*, and in which circulation took place for nine hours. The blastoderm is the same as that from which the section in Plate I, fig. 3 was taken. The vitelline artery, here shown unshaded, has been isolated from the surrounding vessels, which are shaded; *i.v.s.* represents an intervascular strand within the artery; (v_1) and (v_2) are two isolated vascular spaces dorsal to the artery, and (3) represents the plane of the section of Plate I, fig. 3. ×76.

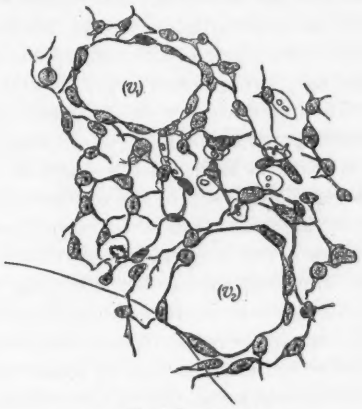
Blastoderms cultivated *in vitro* in which circulation takes place for periods less than that in which arterial differentiation takes place, show an intermediate condition of the mesenchyme. This is illustrated in text-fig. 17, a high-power drawing of the small region of the vitelline artery area of text-

fig. 13, which is drawn from a blastoderm which circulated for four hours *in vitro*. In text-fig. 17, cells are drawn at three different levels, the most ventral at the level of a blood island, next at that of the upper endothelial wall of the vessel network, and most dorsal of all, at that of the mesenchyme above the endothelium. In this mesenchyme, the cells are at moderate distances apart, and the first stages in the formation of the cell processes are to be seen. In the moderately contracted vessels anterior to the vitelline artery area, the cells are much farther apart, and the thickness of the whole mesenchyme is therefore much greater.

Where arterial differentiation has taken place *in vitro*, the vessels of the whole area vasculosa are embedded in a uniformly thick layer of mesenchyme,



Text-fig. 17.



Text-fig. 18.

Text-fig. 17. Drawing of the area (17) of text-fig. 13. Portions (*a*), (*b*) and (*c*) are drawn at different planes. (*a*) represents part of a blood island within the vessels, (*b*) part of the upper vessel wall, and (*c*) part of the mesenchyme dorsal to the vessels. ×400.

Text-fig. 18. Drawing of a region dorsal to the vitelline artery of the twenty-seven-somite blastoderm of text-fig. 16, made before sectioning. In the mesenchyme dorsal to the artery are seen isolated vessel spaces, of which (v_1) and (v_2) are the same as those similarly named in text-fig. 16. Mesenchymal nuclei are shaded, and the nuclei of the dorsal wall of the artery below are unshaded. ×400.

with the cells far apart from each other, and connected by long processes through the intercellular fluid. Under these circumstances it is impossible to draw the vessel network with low magnification from a whole mount; text-fig. 16, representing a differentiated vitelline artery after nine hours' circulation, was obtained by reconstructing from serial sections. In the thick layer of mesenchyme above the vitelline artery, isolated vascular spaces differentiate, as mentioned above. Text-fig. 18 is a high-power drawing, made before the blastoderm was sectioned, of two such vascular spaces which are seen again in the reconstruction (v_1 and v_2); one of them is also shown in the section (Plate I, fig. 3). It was impossible to tell from inspection of the whole mount alone

whether this vascular space had any connection with the artery below, but the absence of such a connection is evident from the section. Occasionally, vascular spaces dorsal to the artery have an open connection with the artery, but where there is such a connection, the space has the same rounded form in cross-section; no typical forms of vascular sprouts from the artery are to be seen, nor does the differentiation of these isolated spaces involve any mitosis in surrounding cells. For these reasons, it has been concluded that these isolated vascular spaces differentiate *in loco* from mesenchyme cells which become endothelial by the conversion of their anastomosing processes into an apparently continuous protoplasmic membrane.

A similar differentiation of isolated vascular spaces above the vitelline artery at the same stage takes place *in ovo*, as will be described below, but *in vitro* the differentiation of the venous plexus dorsal to the vitelline artery from mesenchyme *in loco*, takes place earlier than *in ovo* for blastoderms of the same age, owing to the larger amount of mesenchyme present *in vitro*.

The differentiation of the dorsal venous plexus will not be followed in the present paper beyond its earliest stages. Later, the isolated spaces unite with each other, as in the earliest stages of vasculogenesis, and also with the severed vessels to either side of the vitelline artery. The plexus functions as a means of conveying blood from the hinder regions of the area vasculosa forward to the heart, and does so typically over the vitelline artery first at a point nearest to the embryonic axis, to the left of the embryo. Thoma relates its formation to an increase in pressure in the hinder part of the area vasculosa, where the greater efficiency of the newly formed vitelline artery in conveying blood to that region is not as yet compensated by a more efficient means of venous return than the existing path round the marginal sinus. However, there is first a phase of self-differentiation, as always in the vascular system, which is expressed in the differentiation of the isolated spaces, which appear dorsal to the vitelline artery area. Such isolated spaces are formed also in blastoderms in which no circulation takes place.

Arterial differentiation in blastoderms in ovo

At the time when circulation begins, the main difference between the area vasculosa of a blastoderm *in ovo* and that of a blastoderm *in vitro* is that in the latter the vessels have dilated. We know that *in vitro* when circulation does not take place, all vessels tend to dilate, whereas when circulation is prevented *in ovo*, as in the experiments of Chapman (1918), complete contraction of the vessels ultimately results.

It was concluded above, that in the vitelline artery area there is a slight tendency for the further dilation of the vitelline artery vessels after their first differentiation at the stage of ten somites when they are already of large calibre. By the stage of fifteen somites, some of the intervascular spaces of the vitelline artery area have been reduced still further, some to the condition of intervascular strands, which in some cases have already broken.

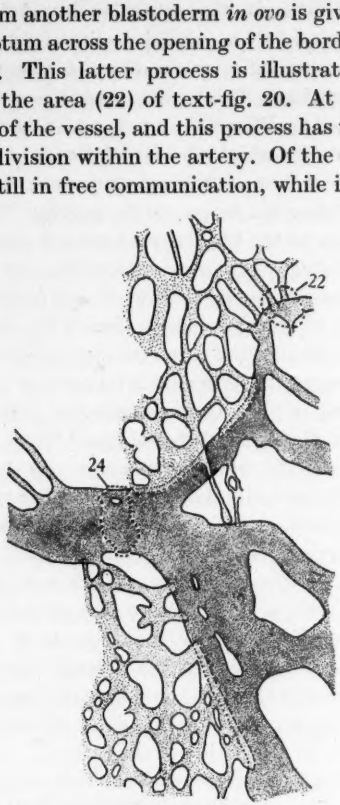
The vitelline artery area when circulation is about to commence is thus a network of vessels of large calibre, some of the intervascular spaces of which are of moderate size, while some are disappearing, as is shown in text-fig. 4. Anteriorly the vitelline artery area leads into a region of vessels of small calibre in virtue of their extension with the embryonic axis as explained above, and posteriorly into vessels of moderate calibre, which have been the last to differentiate. This is the condition of the living vitelline artery area photographed in Plate III, fig. 1, which is from a blastoderm *in ovo* of seventeen somites, in which the circulation had already been in progress for one hour. The next four photographs represent the same vitelline artery area during the succeeding six hours, while text-fig. 19 is a drawing of the same area after fixation of the blastoderm eleven hours after the time of the first photograph at the stage of twenty-four somites, and twelve hours after the beginning of the circulation. In this series we can follow the gradual isolation of the vitelline artery by the increase in size of the bordering intervascular spaces, and the contraction of the corresponding meshes of the network, one of which was seen to disappear. Whereas intervascular spaces to either side of the artery increased in size, those within the differentiating artery became smaller, and finally disappeared in the usual way. Thus the obliteration of intervascular spaces in the vitelline artery area *in ovo* is shared between the pre-circulatory and the post-circulatory periods. In the photographs, the dark areas represent blood islands, which had not been broken up entirely at the time of fixation, and are represented by shading in text-fig. 19.

When the circulation had been in progress for seven hours, that is at the time of Plate III, fig. 5, the flow of blood was markedly more rapid through the differentiating artery than elsewhere, whereas previously the flow had been less unevenly distributed through the area. Two hours later, the circulation was mainly through the differentiating artery, but also in part through vessels running parallel on either side. No blood was passing through the contracted vessels to either side of the artery.

This series represents the first stages in the differentiation of the vitelline artery *in ovo*. No venous return over the artery was observed, and at the end of the period the outline of the artery was still irregular. A later stage in the differentiation of the artery is represented by text-fig. 20, which is drawn from a blastoderm *in ovo* which was fixed at the stage of twenty-six somites, some fourteen hours after the sixteen-somite stage. Here the artery is attaining some regularity of outline, and the dorsal venous plexus proximally is fully differentiated. We shall see later that striking changes occur within the endothelium. In this section we shall refer only to details which can be seen in the differentiation of the dorsal venous plexus. In text-fig. 20 no attempt is made to represent the plexus, but where vessels reaching the artery are cut off from connection with it the line of the artery is continued unbroken, and where vessels pass dorsal to the artery they are represented as cut off just inside the line of the artery.

Isolation of the differentiating vitelline artery can take place in two ways, either by the contraction and obliteration of the lumen of bordering vessels, a process which can be followed in the series of photographs mentioned above, and of which a high-power drawing from another blastoderm *in ovo* is given in text-fig. 21, or by the formation of a septum across the opening of the bordering vessel into the differentiating artery. This latter process is illustrated in text-fig. 22, a high-power drawing of the area (22) of text-fig. 20. At (*s*), a septum has formed across the opening of the vessel, and this process has taken place without apparently causing cell division within the artery. Of the other two vessels joining the artery, one is still in free communication, while in the

Text-fig. 19.



Text-fig. 20.

Text-fig. 19. Drawing of the vitelline artery area of a twenty-four-somite blastoderm, the earlier stages in the differentiation of which are shown in Plate III, figs. 1–5. The shading in the drawing represents blood islands. ×37.

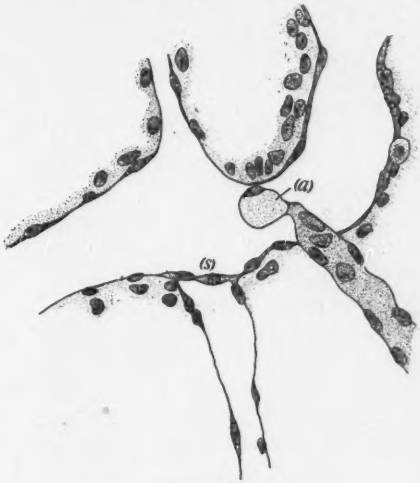
Text-fig. 20. Drawing of the vitelline artery area of a twenty-six-somite blastoderm *in ovo*. The dorsal venous plexus is in process of formation, but is not here shown above the artery. The areas (22) and (24) are drawn at higher magnification in the text-figures bearing those numbers. ×52.

other, in which the endothelium is drawn in surface view, a septum has been formed at a level lower than that which is drawn, and the vessel runs over the artery, to join with an independent vascular space, with which free communication of the lumen has yet to be made. The existence of independent vascular spaces dorsal to the vitelline artery *in ovo* shows that the corresponding phenomenon *in vitro* is of real significance.

Thoma's account of arterial differentiation

The main difference between the outlook of Thoma on the histogenesis of the vascular system and that which is presented here is that since Thoma's time, the contractility of embryonic endothelium has been recognised. The recognition of this property removes some of the difficulties which Thoma encountered, but also suggests the need of caution in interpreting observations on developing vessels, particularly where quantitative treatment is attempted. Thus, Thoma assumed the vessel wall to form an elastic membrane, the tension in which is equal to the sum of the surface tension of the fluid contents and that of the "Zellverbänden getragenen Druckes". The surface tension of a cylinder of fluid is different in the circumferential and longitudinal directions, the

Text-fig. 21.



Text-fig. 22.

Text-fig. 21. Drawing of a small region of the vitelline artery of a twenty-five-somite blastoderm *in ovo*, showing the isolation of the artery by obliteration of the lumen of a bordering vessel. The drawing is made at a level within the lumen of the artery. ×360.

Text-fig. 22. Drawing of region (22) of text-fig. 20. The drawing is made at a level within the lumen of the artery, and shows stages in the isolation of the artery. One side branch has a fully communicating lumen, in another a septum (*s*) has formed across the opening, and in a third, drawn at a higher level, has joined with an independent vessel space (*a*). ×400.

former being twice the latter. Hence Thoma concluded that, in order that the form of the vessel may remain constant, the elasticity of the vessel wall must be different in the two directions. Now, in the first place we have as yet no evidence whether the vessel wall is elastic, and secondly, since the endothelium is contractile, the tension in the wall must depend on the state of contraction over and above the effect of physical forces.

To Thoma's description of the area vasculosa at the stage when circulation begins we have been able to add only a description of the means whereby the

narrow calibre of the vessel proximal to the middle somites is attained, namely by their extension with that of the embryonic axis. Also the present account of the gross anatomical changes which take place in the differentiation of the artery is in agreement with that of Thoma, except for differences in detail. His high-power drawings, however, do not take us into this period, and stop at the stage of the pre-circulatory differentiation of the network, although he has noticed the cell division in the wall of the differentiating artery which as described below follows on its growth under the influence of the circulation.

Thoma interprets this influence as essentially a nutritive one. "Die vom Blutstrome bevorzugten, rascher durchströmten Capillaren wachsen stärker und werden demgemäss zu weiteren Röhren als die minder reichlich gespeisten Capillaren". Roux (1879) in his classical work on the angle of branching and form of cross-section of an arterial system in equilibrium with the blood flow which the system was transmitting, showed that the physical forces generated by the blood stream were the essential factors in moulding the form of the arteries, and that the form assumed was such as to offer least fluid resistance to the stream. It is therefore interesting to enquire how soon after the development of the circulation do physical forces play a part in the moulding of the vascular system, and what is the nature of the means through which this influence operates. In the succeeding section, evidence is given that physical forces generated by the blood stream play their part in the moulding of the vitelline artery. This evidence is based on the elongation of the endothelial nuclei which takes place in the wall of the differentiated artery, and its effect on the mitotic spindle at division. It is suggested that nuclear elongation is an index of tension action within the cell, and that tensions in the wall of the artery are set up by the fluid friction generated by the blood flow.

It can here be stated, however, that on general principles it seems improbable that a blood vessel through which blood is flowing rapidly should enjoy better nutritive conditions than one in which the flow is slower. Were this so, the endothelium must extract its nutritive substances from the blood stream almost instantaneously. Moreover, it would have to be possible for adjacent areas of endothelium to enjoy very different nutritive conditions without influence on each other. When the artery is differentiating, its lumen is enlarging, whilst the diameter of adjacent vessels is contracting, a process which we have spoken of as the isolation of the developing artery. Now isolation does not only take place by the narrowing of the bordering vessels, there is also the formation of septa across their openings into the artery to be considered, which is the process illustrated in text-fig. 22. This is incomprehensible on a purely nutritional explanation.

This leads us to the general account of the formation of the dorsal venous plexus which is given by Thoma. He states that the plexus is first formed proximal to the embryo, as described above and during its formation, "Gefässneubildung" and "Gefässschlund" go on side by side. He did not observe, however, the formation of those isolated vascular spaces dorsal to the artery, which

join up with the severed vessels, and thus the self-differentiating aspect of the formation of the dorsal venous plexus was obscured in his account. This aspect is further emphasised when isolated vascular spaces are seen dorsal to the vitelline artery area when no circulation takes place at all.

The formation of the dorsal venous plexus, according to Thoma, is in relation to an increase in pressure in the small vessels in the hinder part of the area vasculosa, which takes place when the artery has differentiated, and the resistance offered to the circulation on its way to this region is diminished in consequence. This excess pressure is relieved when the dorsal venous plexus is formed, which functions as a correspondingly more efficient means of venous return for this region. Thoma links this with the third of his histomechanical principles, which is put forward on a different footing to that of the other two, since it is left as a hypothesis. Thoma's three histomechanical principles are as follows:

(1) The increase in size of the lumen of a vessel, which is proportional to growth in surface of the vessel wall, is dependent upon the rate of the blood flow.

(2) The growth in thickness of the vessel wall is dependent upon the tension in the wall, which in turn is determined by the diameter of the vessel, and by the blood pressure.

(3) Increase of blood pressure above a certain limit, which limit is defined by the metabolism of the particular tissue, leads to the new formation of capillaries.

The first of these principles is incontestable, and has been proved by Thoma both in the work on the chick blastoderm, and in his earlier researches on pathological material. The second principle in its application to the chick area vasculosa falls outside the stages studied by Thoma and by the present writer, but the fact that increase in blood pressure leads to a thickening of the tunica media was proved by Thoma's earlier work, and also by the experiments of Fischer and Schmieden (1909), who transplanted a length of a vein into the path of an artery, and observed a great increase in thickness of the tunica media of the implanted vein.

The third histomechanical principle was formulated entirely from the work on the chick blastoderm. Thoma maintained that the extent of the initial vascularisation of an organ depended on the metabolism of the tissues of that organ, and the fact that some organs are more vascular than others was regarded as evidence for this view. Later increase in blood pressure can, however, increase the density of vascularisation. It is certainly true that the area vasculosa does increase in size, and that as it does so, new formation of capillaries goes on at such a rate that the average size of the intervascular spaces steadily diminishes. It is also true that during the first few days of circulation the output from the heart steadily increases. However, the possibility of a causal connection between these two facts was destroyed by Chapman, who showed that when no circulation takes place, the area vasculosa continues to increase

in size, although not so regularly as normally; that the new formation of capillaries takes place at about the normal rate is shown by inspection of Chapman's figures of the injected area vasculosa which has continued to grow when deprived of the circulation.

The fact concerning the relations between self- and functional differentiation of the vascular system which should be elevated into a histomechanical principle is the following: in a vascular system, the regular tubular form of the vessels is only indefinitely maintained when blood continues to circulate through them, although if the circulation ceases the endothelium persists as a tissue different from other mesoderm after the tubular form of the vessels is lost. This was demonstrated by Chapman's experiments, in that while new formation of vessels goes on at the periphery of the area vasculosa without circulation, those at the centre contract and undergo regression. Where isolated whole organs such as limb-buds are cultivated for a considerable time *in vitro*, it is easy to see that while the endothelium within the explant persists as a definite tissue, it no longer takes the form of a system of tubes. Some vessels expand into sinuses, while others are reduced to strands of cells without a lumen.

Results which have been obtained in the course of the present work from the cultivation of fragments of area vasculosa will be described in the succeeding subsection. They illustrate further the influence of the shape of a vessel on the form of the endothelial nuclei.

V. THE FORM OF THE ENDOTHELIAL NUCLEI

So far our account of vascular differentiation has been limited mainly to a description of the changes in the gross anatomy of vessels which can be seen with low magnifications. It remains to consider whether these changes can be further elucidated by the study of the individual cells which compose the walls of the vessels.

As the cell boundaries in embryonic endothelium are usually not seen, the account of the changes which take place in the endothelial cells is limited to the alterations in shape of the endothelial nuclei.

Moreover, since the tunica media of the extra-embryonic arteries does not develop until considerably later than the period of the first differentiation of the artery with which this paper is concerned, these observations on the shape of the endothelial nuclei apply solely to the period when the wall of the artery consists only of a single layer of endothelial cells.

The endothelial nuclei and vascular differentiation

The purpose of this section is to describe the relationships which exist between the histogenesis of vessels, the form of the endothelial nuclei, and the relative amount of cell division which takes place at each stage. The mesoderm of the area vasculosa which has migrated out from the primitive streak at a stage prior to the first appearance of vessel spaces, consists of strands of cells in close contact with each other, in which cell boundaries are readily recognised.

The nuclei are spherical or ovoid, and everywhere mitotic figures are exceedingly abundant. When the first isolated vascular spaces appear, the form of the cells does not immediately change, and mitosis continues as before, but later the cells differentiate into two types. The cells round the vascular spaces which form the first endothelium flatten, and cell boundaries are no longer distinguishable with ordinary cytoplasmic stains, while elsewhere cells become mesenchymatous in that they then consist of separate cell bodies united by anastomosing processes. The views of various authors differ on the exact method whereby the endothelial cells become flattened; in this section only the changes which take place in their nuclei will be described. The endothelial nuclei when seen in surface view remain rounded or oval, but become larger in diameter, and less deeply staining; when seen from the side it is apparent that they have flattened, and their lighter stain is due to their thinness. This is the stage of the fusion of the now enlarged contiguous spaces, and mitotic figures are still plentiful, though less so than before. When division of flat endothelial cells takes place, the nucleus remains flat when the nuclear membrane disappears, and in late prophase, the chromosomes are spread out over an area larger than that of the non-dividing nucleus, whilst the plane of the equatorial plate is at right angles to that of the vessel wall. During the later stages of division the cytoplasm of the dividing cell is thicker than that of the surrounding cells, and the cell boundary is easily distinguishable.

When the vessel network has everywhere a patent lumen and is fully differentiated, mitosis gradually diminishes, until by the time the sixteen-somite stage is reached, there are comparatively few mitoses throughout the endothelium; cell division still goes on in the blood islands however, where pycnotic nuclei are also frequently found, while in the endothelium nuclear degeneration is hardly ever seen at any stage.

An exception to the rule that with the full differentiation of the vessel network there is a great reduction in cell division is afforded by the vitelline artery vessels. They are, as explained above, of large calibre from their first differentiation at the stage of ten to eleven somites, and at that time (text-fig. 3) there are still numerous mitotic figures present, which, however, have become fewer in number by the sixteen-somite stage, by which time the endothelial nuclei in the vitelline artery vessels are closer together and more numerous than elsewhere. During the first few hours of circulation, and before there has been any effect on the anatomical disposition of the vessel network, cell division in the vitelline artery vessels falls to the same low level as that elsewhere in the vessel network, and at this time there is less cell division throughout the area vasculosa than at any other period.

When vessels increase in length, at first no cell division takes place in the wall, and the endothelial nuclei become farther apart. It has been explained above how the vessels in the area pellucida opposite the middle somites extend parallel to the embryonic axis with the increase in length of the embryo. At ten somites (text-fig. 2) this extension has begun, but there are still some mitotic

figures in the endothelium which represent the last traces of the cell division accompanying differentiation. At sixteen somites (text-fig. 4), although the shape of the intervascular spaces in this area implies a great extension of the vessel network, there is hardly a mitotic figure to be found in the vessel walls, and the endothelial nuclei are farther apart from each other than elsewhere in the vessel network.

Meanwhile, the area vasculosa is increasing in size owing to the increase in circumference of the marginal sinus, and the first effect of this process within the vessel network is a progressive increase in length of the network meshes. Something of this process can be seen in Plate I, figs. 1 and 2, photographs of the same vitally injected blastoderm. Not until the area vasculosa has increased considerably in size is this extension of the vessel network correlated with the appearance of endothelial cell division. This occurs at about the stage of twenty-six somites.

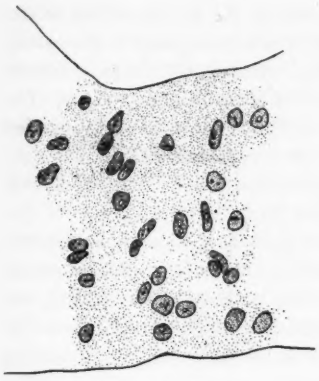
Another gross change which can take place in the vessel network without being accompanied by mitosis is the dilation *in vitro* in the absence of a circulation. A high-power drawing of this process is given in text-fig. 6; the endothelial nuclei are far apart, and mitotic figures are very rare.

The changes in the vitelline artery area due to the maintenance of the circulation, namely, the differentiation of an artery, are not at first accompanied by cell division within the endothelium. The difference in gross appearances between arterial differentiation *in vitro* and *in ovo* is reflected in the extent to which cell division takes place in the endothelium in each case. As mentioned above, the stage at which the circulation normally begins is one at which mitosis is at a minimum throughout the vessel network. When a blastoderm has been explanted some time previous to this stage, the extra-embryonic vessels are generally more dilated than *in ovo*, and the absence of mitosis in the endothelium corresponds to that seen in dilation where no circulation takes place. After the first few hours of circulation vessels other than those of the vitelline artery area contract somewhat, and in the dilated vitelline artery area occasional mitotic figures now appear. In text-fig. 17, a high-power drawing of a portion of the vitelline artery area of a blastoderm in which four hours' circulation *in vitro* took place, only one prophase is to be seen in the area of endothelium drawn. In the vitelline artery area drawn in text-fig. 14, where six hours of circulation took place, mitotic figures are slightly more numerous than before. The isolated artery after nine hours of circulation *in vitro* consists of a tract of dilated vessels with contracted vessels to either side. In the dilated vessels which form the artery, the endothelial nuclei are still far apart, and this condition is maintained even after twenty hours' circulation. Mitotic figures in the endothelium never become very numerous. In text-fig. 23, a drawing of an area of the vitelline artery of a thirty-somite blastoderm in which thirteen hours' circulation *in vitro* took place, there were no mitotic figures in the area chosen.

The differentiation of the artery from dilated vessels *in vitro* is to be con-

trasted with arterial differentiation *in ovo*, where the vitelline artery area is not dilated and contains some large intervascular spaces. In the photographs (Plate III, figs. 1–5) it can be seen that the vessels which differentiate into the vitelline artery are more contracted after their differentiation than before. In this blastoderm (text-fig. 19) the vitelline artery after twelve hours' circulation is of irregular outline, and in its wall there are very few mitotic figures.

Large numbers of dividing cells first appear in the wall of the vitelline artery *in ovo* at the stage when the dorsal venous plexus is in process of formation, and the artery is acquiring a regular outline. This stage is reached usually when the embryo has twenty-six somites, fourteen hours from the time when



Text-fig. 23.

Text-fig. 24.

Text-fig. 23. Drawing of a region of the upper wall of the vitelline artery of a thirty-somite blastoderm, cultivated *in vitro*, in which circulation took place for thirteen hours. This field is exceptional in showing no mitotic figures. ×360.

Text-fig. 24. Drawing of region (24) of text-fig. 20. It represents this part of the upper endothelium of the artery. ×400.

circulation first begins, and the relative number of dividing cells in the wall of the artery at this time is illustrated in text-fig. 24, a high-power drawing of the area (24) of text-fig. 20. In comparing blastoderms *in ovo* between the stages of twenty-three and twenty-six somites, it is evident that the artery when first differentiated must offer as much resistance to the circulation as did the vessels from which it has been formed, for as yet there has been no enlargement of the artery (Plate III, figs. 1–5, text-fig. 19). If the effectiveness of the heart beat and the force of the circulation continually increases during the first twenty-four hours of circulation, as is certainly suggested by inspection, then

in the period immediately before growth of the differentiated artery begins, the frictional resistance offered by the whole vitelline artery area must then be at a maximum. During the next few hours, the artery increases in diameter comparatively rapidly, and cell division within the wall begins, and more than makes up for the increase in diameter of the artery, so that in the end the endothelial nuclei in the arterial wall are closer together than ever before.

The vitelline artery after differentiation *in vitro* remains in the dilated condition of vessels from which it has been formed, and is thus comparatively large in diameter from the beginning, and at its first differentiation the frictional resistance which it offers to the blood stream cannot be maximal. Accordingly, the endothelial nuclei within the wall of the vitelline artery *in vitro* remain comparatively far apart from each other, and much less cell division takes place than in the vitelline artery *in ovo* in the first few hours after differentiation.

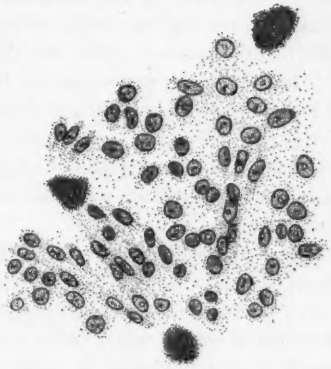
We have now to consider a related phenomenon in the fine structure of the vessels, which sheds further light on the changes which take place in the artery after the first differentiation. This phenomenon concerns the shape assumed by the non-dividing nuclei, and the part which this plays at cell division. The nuclei in differentiated endothelium are flattened, but when seen in surface view are generally rounded or oval. However, when vessels are either strongly contracted or widely dilated, the endothelial nuclei tend to be distorted from their rounded shape, and to become elongated. In contracted vessels the elongation of the endothelial nuclei is along the axis of the vessel, and takes place wherever vessels of small calibre are found. Such are the sprouts which grow out from the first formed vessels in the area pellucida (text-fig. 1), the elongating vessels in that region of the area pellucida which extends with the growth in length of the embryo (text-fig. 20), or the contracted vessels bordering on the differentiating vitelline artery, which either contract until the lumen is obliterated (text-fig. 21), or are cut off from the artery by the formation of a septum.

Where the intervascular spaces are of medium size, and the vessels are moderately dilated, all the endothelial nuclei are either rounded or oval, and the range of vessel calibre over which this holds good extends from the moderately contracted vessels of the area pellucida opposite the middle somites at the sixteen-somite stage, to a degree of dilation rather less than that of the vitelline artery vessels at the stage of ten somites (text-fig. 3), when nuclei begin to elongate in response to dilation. Where vessels dilate *in vitro*, nuclear elongation is observed at the stage in the process when the intervascular spaces are becoming so small that they are forming intervascular strands, when the elongation tends to be along lines joining these intervascular strands. Text-fig. 25 is a drawing of a small area of the vessel network of a blastoderm which was cultivated *in vitro* without circulation taking place for a period of seven hours after the sixteen-somite stage, and shows elongation of endothelial nuclei in this manner. A similar elongation is seen in blastoderms in cultivation where circulation has taken place. There is some nuclear elongation in the

vitelline artery area of the blastoderm in which circulation took place for four hours which is drawn in text-fig. 17, but this is more extensive when circulation has been in progress for six hours. With the differentiation of the vitelline artery, nuclear elongation becomes more pronounced, as is shown in text-figs. 18 and 23.

Elongation of the endothelial nuclei also accompanies arterial differentiation *in ovo*. It is less marked in the irregular artery at twenty-four somites than at twenty-six somites when the outline of the artery is regular, and when mitotic figures have appeared in large numbers in the endothelium (text-fig. 24).

At twenty-six somites (text-fig. 24), at which stage both elongation and mitosis are considerable, the endothelial nuclei are frequently elongated in a direction at right angles to the axis of the artery. Not all the elongated nuclei point in this direction however, and a general trend for orientation to be at right angles to the axis of the vessel is only seen where the artery has the form of a straight tube without side branches. Where the individual nuclei are most elongated, that is with a ratio of length to breadth of 2·5 : 1, all the surrounding nuclei tend to be orientated in the same direction within the endothelium, but the direction of orientation of nuclei immediately overhead within the mesenchyme may be quite different.



Text-fig. 25. Drawing of a region of the upper wall of the vitelline artery vessels of a twenty-two-somite blastoderm cultivated *in vitro* without circulation. Complete dilation of the vessels took place, and the three dark areas represent intervascular strands. There is a tendency for the elongation of endothelial nuclei between these strands. × 360.

Endothelium in tissue culture

In the preceding subsection, an account was given of the influence of the form of vessels in the area vasculosa on the shape of their endothelial nuclei. The personal observations described in this section refer to the different forms assumed by vessels which outwander from explants of fragments of area vasculosa in tissue culture, and their relation to the shape of their endothelial nuclei.

The cultivation of vascular endothelium *in vitro* has been frequently described, and the literature relating to this subject has been reviewed by Levi (1934) in his survey of the whole subject of tissue culture.

Most of the earlier accounts refer to the outgrowth of endothelium not in the form of vessels but as a plexus of cells, which under favourable circumstances can be distinguished from other types of mesodermal cells. In general, the outgrowth of endothelium comes from vessels present in the explant, and only in the work of Rienhoff (1922) and of Murray (1932) was an actual dif-

ferentiation of endothelium observed, which in both cases assumed the form of hollow vessels. Rienhoff explanted fragments of embryonic metanephros, and obtained the differentiation of sinus-like vessels, while Murray explanted fragments of chick primitive streak, which underwent a differentiation into blood cells and vessels similar to that undergone by the primitive streak mesoderm *in vivo*.

Where the outgrowth of hollow vessels is obtained as in the work of W. H. Lewis (1922), and of Scriba (1935), the form of the endothelium as hollow tubes is never indefinitely maintained, but the extension of the vessels in the zone of outgrowth leads to the breaking up of the lumen into isolated spaces, and finally to the outwandering of the individual endothelial cells in a fibroblast-like form (Ephrussi, 1930; W. H. Lewis, 1931; Scriba, 1935).

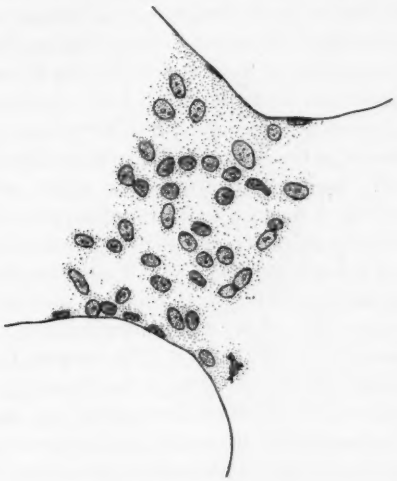
In the present investigation, fragments of area vasculosa were explanted at a stage subsequent to the differentiation of the vessel network, so when an outgrowth of vessels was obtained it had come from pre-existing vessels. The embryos used ranged from the primitive streak stage to that of twenty-two somites, and explants were taken from the area pellucida, or in one experiment from the area opaca.

Out of eighty-four cultures, only seven showed outgrowing vessels in the form of hollow tubes. When the outer wall of the exocoel was not dissected off, as in the majority of the experiments, the culture formed a rounded cyst, the interior of which, when vessel outgrowth took place, became lined with a network of vessels similar to those of a whole area pellucida. Usually, however, comparatively little new formation of vessels occurred; the original vessels of the explant dilated as previously described in the case of explants of the whole area vasculosa in the absence of circulation, and finally all that was visible were the long intervascular strands which represent the intervascular spaces when complete dilation takes place.

In one experiment in which area opaca was explanted at the ten-somite stage, the outer coelomic wall was dissected off, and the endoderm spread out as a flat epithelium. In one culture out of ten, new vessels sprouted from those of the original explant forming a network of vessels over the surface of the endodermal outgrowth, as shown in Plate III, fig. 7. The vessels in this photograph are conspicuous owing to their heavy content of erythroblasts, formed presumably by multiplication of the cells of the blood islands of the original explant. The vessels in Plate III, fig. 7 have not dilated, for the outgrowth of vessel sprouts has been so extensive that the lumen has everywhere collapsed. The upper and lower walls of the vessel network are only separated by a layer of erythroblasts, many of which are degenerating. A drawing of the area (26) of Plate III, fig. 7 is shown in text-fig. 26; it can be seen that the large extension of the vessel network and the obliteration of the vessel lumina are reflected in the shape assumed by the endothelial nuclei, which have become very large, and tremendously elongated in the direction of the length of the vessel. Their ratio of length to breadth is, in some cases, as much as 4 : 1.

In another series of cultures which were made from fragments of the area pellucida of a twenty-two-somite blastoderm, extensive outwandering from the whole explant took place, and the mesenchyme surrounding the vessels migrated into the medium as cells with the typical form of tissue culture fibroblasts. On examination of the fixed culture it is seen that the vessels of the explant, which are identified by their content of degenerating blood vessels, no longer possess definite walls, but the nuclei of the cells bordering the lumen are elongated in the same direction as those of the surrounding fibroblasts. It appears that the endothelium has here differentiated into fibroblast-like cells.

Text-fig. 26.



Text-fig. 27.

Text-fig. 26. Drawing of the area (26) of the culture of area opaca of Plate III, fig. 7. The large nuclei within the vessel are endothelial, and the smaller darker nuclei are those of erythroblasts. × 400.

Text-fig. 27. Drawing of a region of the upper wall from a vessel from a network in a cyst-like culture of area pellucida, in which the vessel lumina remained of normal dimensions. × 360.

Where extensive outwandering of the vessels of the explant is prevented by the formation of a cyst, the vessels usually dilate eventually, but before this stage is reached normal tube-like vessels can be seen. Text-fig. 27 is a drawing of such vessels in a culture in which the lumen is still of moderate dimensions. It will be noticed that nuclear elongation in the wall at this stage is not extensive.

There is therefore considerable evidence that the form of vessels as uniform tubes is not indefinitely maintained in the absence of a circulation. De-differentiation of the endothelium does not necessarily follow the loss of the external form of a vessel, but where alteration of external form occurs then the shape of the endothelial nuclei reflects the changes which take place.

Nuclear elongation as an index of tension, and its effects on mitosis

It has been shown above that the alteration in the form of a vessel from that of a uniform tube of moderate diameter to one with a smaller or larger lumen is accompanied by elongation of the endothelial nuclei in particular directions depending on the nature of the change. In this section, evidence is considered which suggests that such nuclear elongation is an index of mechanical tensions acting on the cell.

Most of the evidence which has accumulated within recent years regarding the consistency of the living nucleus indicates that the nuclear contents are of a fluid nature, while the nuclear membrane is solid (Chambers, 1924; Gray, 1927). Consequently we should expect that a living nucleus would be deformable by physical forces. That endothelial nuclei can change their shape comparatively rapidly can be demonstrated by causing the vessels in a region of an area vasculosa to contract. A number of blastoderms were cultivated *in vitro* after removal of the embryo. The vessels dilated, with the result that the endothelial nuclei were comparatively far apart, and displayed no general distortion. Text-fig. 6 shows a part of such an area vasculosa. One of these blastoderms with dilated vessels was then injected with the Indian ink that was used in the vital injection method, which was described in the second section. The injection caused the vessels to contract, and the precipitation of the ink on the inner surface of the vessel wall rendered visible the condition of the vessel network as shown in Plate III, fig. 6. The vessels at injection were in the same condition as those shown in text-fig. 6, but began to contract a few minutes later. Thirty minutes after injection, the contraction had become more severe, and this condition persisted at fixation, ten minutes later. Text-fig. 7 is a drawing of part of this blastoderm under high power, and it can be seen that there has been a general tendency for the endothelial nuclei to elongate. Now when the endothelium contracts it does so in an isometric manner, since the distance between the mid-points of the intervascular spaces remains approximately the same, and the tension developed within a mesh of the vessel network may be considered proportional to the length. Where the circumference of a contracted vessel is less than the length, we should expect that the lines of maximum tension will be along the vessel.

Now nuclear elongation is uniformly along the axis of the vessel in the longer meshes of the network, as shown in text-fig. 7, and the only sites where elongation is not uniform are at the nodes where several vessels meet. This is precisely what would be expected on the hypothesis that nuclear elongation is an index of tension.

The next question to be considered is whether there is any effect on the orientation of the mitotic spindles in an area of endothelium or epithelium in which the resting nuclei show a general elongation in one particular direction. For this purpose, the area vasculosa of a blastoderm cultivated *in vitro* without circulation will not serve, for there is very little mitosis in the endothelium. In

early development, the most striking example of a tissue in which both general nuclear elongation and mitosis occur simultaneously is the endoderm of the area pellucida of blastoderms *in ovo* during the formation of the first ten somites. Here, nuclear elongation at the edge of the area pellucida in a direction parallel to the border of the area opaca is a very marked feature, and the evidence for the influence of this elongation on the direction of the mitotic figures is presented in the accompanying Table II. That the area pellucida tends to be under a state of tension is indicated by its behaviour when a portion of a blastoderm is explanted *in vitro*, when contraction takes place along a cut edge of the area pellucida.

Nuclear elongation in the pellucida endoderm is much more marked at the margin than near the embryo, where the endodermal nuclei are generally rounded. Where in the margin the elongation is generally greatest, there the axes of the division spindles are almost exclusively in the direction of the elongation. Such an area is illustrated in text-fig. 28. The elongation of the non-dividing cells is seen to affect not only the nucleus but also the cytoplasm, which is much denser opposite the two ends of the extended nucleus. Where elongation is less pronounced, the number of mitotic spindles in line with the general elongation still predominates, but a certain proportion are at right angles to the direction of elongation. In Table II the dividing nuclei are classified under three headings, apart from prophases which although usually extended in the direction of elongation, offer no certain indication of the direction of the future spindle. The three categories depend on the angle at which the spindle is orientated with relation to the margin of the pellucida.

Text-fig. 28. Drawing of a region of the endoderm of the area pellucida of a seven-somite blastoderm at the boundary between area pellucida and area opaca. The outlines are those of spaces within the mesenchyme dorsal to the endoderm. (*m*), area opaca margin. ×400.

From the results given in Table II, it can be seen that in areas of general nuclear elongation, the number of mitotic spindles within 15° to either side of the general direction of elongation is nearly twice the number of those occupying the remaining 150° of the half circle. There can therefore be no doubt that nuclear elongation influences the direction of the mitotic spindle in these particular tissues, and presumably also in all unilayered pavement epithelia.

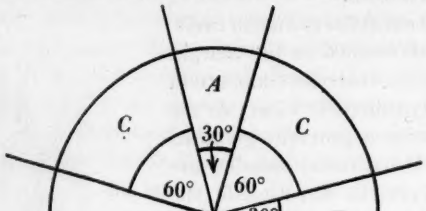
A correspondence between nuclear elongation and the direction of the mitotic spindle is also seen very clearly in the fibroblast-like cells which outwander into the medium from an explant of embryonic chick heart, when a speeded-up cinema film of such a culture is observed. This correspondence has

been incidentally investigated by Paul Weiss in his work on the cultivation of fibroblasts on a film of medium coagulated across a small triangular or oblong frame (1929, 1933). In such cultures, the outgrowth is most abundant along certain directions, which, according to Weiss, are the lines along which the micellae of the plasma coagulum are orientated by the tensions set up within

Table II.

Nuclear Elongation and Mitosis

The mitotic figures in areas of nuclear elongation are classified into four groups; prophases, and later stages according to the axis of the spindle into the groups (A), (B) and (C) as follows:



The Area Pellucida Endoderm

Nuclear elongation is seen at the opaca border in a direction parallel to the boundary between pellucida and opaca.

Blastoderm	Prophases	A	B	C
3 somites	7	22	7	8
7 somites (text-fig. 27)	17	45	12	21
11 somites	4	25	6	5

The Vessels of the Area Pellucida

At ten somites onwards, the vessel network in the area pellucida extends as a whole with the embryonic axis, and nuclear elongation is seen in the endothelium parallel to the axis.

	Prophases	A	B	C
Vessels in the pellucida in an area opposite somites five to eleven in an eleven-somite blastoderm (text-fig. 8)	1	18	4	8

the coagulum either during clotting, or by the weight of the culture, or by the abstraction of water by the culture. Where outgrowth is most luxuriant, the long axes of the fibroblast nuclei all tend to be orientated in the same direction as that of the general outgrowth, and when division takes place, the mitotic spindles are similarly arranged. Nuclear elongation tends to be more pronounced in the orientated region, and the mitotic figures more abundant.

Weiss explains his results not as the result of tension acting directly on the cells themselves, but as the result of their tendency to grow along orientated protein micellae. The pattern of the culture reflects the invisible pattern of the "ultrastructures" within the plasma film.

The main importance of Weiss' results in relation to the present study is his observation of the correlation between nuclear elongation and the direction of the spindle at division. His explanation, however, although possibly correct for his cultures, can hardly be applied to such a case as the area pellucida endoderm, where a whole circular tract of epithelium undergoes a general extension. In these endodermal sheets we are far more likely to be dealing with the effect of tension on the cells themselves.

In prevascular "einreihige Zellsträngen" (Rückert and Mollier, 1906), there is elongation of the nuclei in the direction of the whole strand, and the axes of the mitotic spindles also lie in this direction. In the vessels opposite the middle somites at the ten-somite stage, when extension of the whole network in this region keeps pace with that of the embryonic axis, many of the endothelial nuclei are elongated parallel to the embryonic axis, and the endothelial mitotic figures show a strong tendency for a similar orientation. In Table II, a count is given of the mitoses in the specified region of the area pellucida of the eleven-somite blastoderm of which Plate II, fig. 6, is a photograph, and text-fig. 2 a high-power drawing of the area (*b*).

Where embryonic vessels elongate, therefore, the orientation of the endothelial mitotic figures tends to correspond with the direction of the elongation of the resting nuclei. But the present study is primarily concerned with arterial differentiation within the vessel network, and we must therefore enquire whether the nuclear elongation within the differentiated artery has a similar influence on the endothelial mitotic spindles.

In ovo, when the differentiation of the artery reaches the stage shown in text-fig. 20, at which the dorsal venous plexus is in process of formation and the outline of the artery is becoming regular, nuclear elongation for the first time becomes a marked feature of the endothelium, and at the same time mitotic figures increase in number. Although many of the endothelial nuclei are elongated at right angles to the axis of the vessel, others are elongated in other directions, and there is no general preponderance of mitotic spindles at right angles to the axis of the vessel.

If it is true that the nuclear elongation which accompanies the differentiation of the vitelline artery is due to physical forces generated by the circulation, the wall of the vitelline artery should be under tensions which would be relieved if the flow of blood were interrupted. Experiments were therefore made in which the whole posterior region of an area pellucida was explanted at the stage of thirty somites, when there is a fully differentiated dorsal venous plexus over the proximal part of the vitelline artery; it was found that whereas the vessels of the dorsal venous plexus dilated as do other vessels of the area vasculosa under similar conditions, the vitelline artery did not dilate, but slowly contracted to a

thin cord, which was made visible by reason of its content of blood cells. Text-figs. 29 and 30 are camera lucida drawings of a living explant of the hinder part of an area pellucida at thirty somites and Plate I, fig. 5, is a photograph of a section through the explant, which was fixed ten minutes after text-fig. 30 had been drawn. The section shows that much degeneration took place in the blood cells within the artery, and in the mesenchyme round the vessels, but not in the endothelium itself, or in the walls of the extra-embryonic coelom. As far as can be judged from sections, the nuclei in the wall of the vitelline artery are no longer elongated. The dilated venous plexus is seen dorsal to the contracted artery.

If the hinder part of the area pellucida is explanted at an earlier stage, either before the process of isolation of the artery is completed or when the isolated artery is still irregular in outline, dilation occurs, and not contraction, as in the case of the regular artery in which nuclear elongation and mitosis are taking place in the wall. Correlated with the nuclear changes in the wall of the artery is a change in the reaction of the whole vessel to the cessation of the blood flow.

Text-fig. 29.

Text-fig. 30.

Text-figs. 29 and 30. Drawings of the vitelline artery area of a thirty-somite blastoderm, in which the posterior region was isolated and cultivated *in vitro*. The vessels of the dorsal venous plexus dilated, while the vitelline artery contracted. Text-fig. 29 was drawn seven hours after explantation, and text-fig. 30 ten and a half hours after. The vitelline artery is shaded. ×24.

If we apply the results shown in Table II to the vitelline artery at twenty-six somites, and assume that when an elongated nucleus divides, the axis of elongation and the axis of the spindle will correspond, we shall be postulating a mechanism for the relief of tensions within the endothelium which will give rise to a directional growth. The tensions are set up within the endothelium by the fluid friction generated by the circulation, and directional growth will mould the form of the vascular system to that which will offer minimal resistance to the flow of blood, which is the case in a fully formed arterial system, as Roux concluded.

Thoma, however, inclined more to the view that nutritional factors were those responsible for the first phases of arterial growth, for the reason that he could see serious objections to ascribing arterial growth to the influence of fluid pressure. Certain equally serious objections to his nutritional hypothesis are mentioned above, and since Roux has shown that fluid friction is the main force at work in the later functional differentiation of the vascular system, it is natural to enquire whether this force does not operate at earlier stages of vascular development.

Many points will have to be investigated further in such an inquiry. In particular, the exact relation between the operation of chemical and physical factors in causing mitosis must receive attention. It is obvious that tension

acting on a cell cannot be regarded as the sole cause of mitosis, for nuclear elongation can be found in areas where no cell division takes place, and, in general, cell division is known to be stimulated by chemical factors. It may be that when cell division appears in the vitelline artery there is a change in its chemical environment which induces cell division; it is interesting to notice in this connection that the period when mitoses are first abundant within the artery is also the period when cell division in the general network occurs in response to the increase in size of the area vasculosa. One further point in relation to the special problems of the form of the vascular system is whether nuclear elongation can always be regarded as an index of tension, in particular after the development of the tunica media, when the endothelial nuclei of the arteries are all elongated in the direction of the length of the vessel.

That the physical aspects of the blood stream are important in moulding the shape of the first differentiated artery is strongly suggested by the alteration in cross-section which the artery undergoes during the first day after the beginning of the circulation. The change is from the flattened and somewhat irregular cross-section seen in Plate I, fig. 4, to the uniformly circular one seen in Plate I, fig. 6, which is taken from the vitelline artery of an embryo of thirty-three somites, twenty-seven hours after the circulation began.

VI. SUMMARY OF RESULTS

1. There are two periods in the differentiation of the area vasculosa. First, a period of self-differentiation before the circulation begins during which a more or less uniform network of vessels develops, and secondly, a period of functional differentiation during which arteries and veins differentiate within the network. This paper deals only with the first example of visible functional differentiation in the area vasculosa, namely the differentiation of the vitelline artery.

2. In the period of self-differentiation, the path along which the blood will mainly flow can be recognised some hours before the flow actually begins. This path is made up of vessels of larger calibre than elsewhere in the network, and when the flow begins, offers less frictional resistance than other alternative paths.

3. Vessel formation in the extra-embryonic mesoderm begins with the differentiation of isolated vascular spaces, which enlarge and then join up to form the vessel network. Where the first vessel spaces are far apart, they are joined by means of outgrowths from the spaces known as "sprouts". In this way a network of vessels of small calibre is formed.

4. Embryonic vascular endothelium is a contractile tissue; in general it dilates when small stimuli are applied, and contracts when strongly stimulated. When blastoderms are explanted *in vitro*, a dilation of the vessels takes place before the circulation begins, and persists should circulation not occur. One effect of the circulation *in vitro* is to stimulate the vessels to contract.

5. When vessels fuse, two layers of endothelium come into contact, and

are then resorbed. This is seen in the fusion of the first isolated vessel spaces, in the fusion which takes place when dilation *in vitro* persists, and where vessels fuse together in the formation of the artery. In the two last examples, the process leads to the disappearance of round intervascular spaces within a vessel network.

6. When circulation occurs *in vitro*, the process of differentiation of the vitelline artery can be followed. After six hours' circulation, the vessels outside the area in which the artery will form are less dilated than they would be if circulation had not occurred, but no other change has yet taken place.

7. The artery is differentiated after nine hours' circulation *in vitro*. It is formed proximally from a tract of the vessel network which, as shown by the intervascular spaces within it, is two or three network meshes in width. The differentiation is mainly effected by the disappearance of the connections between the differentiating artery and the smaller vessels to either side. This process is spoken of as isolation.

8. The vessels of the area vasculosa *in vitro* are more dilated than *in ovo* during the period before the differentiation of the vitelline artery. The vessels of the region in which the vitelline artery will differentiate are more dilated than those elsewhere in the area vasculosa both *in ovo* and *in vitro*. The vitelline artery is isolated at the same time after the beginning of circulation both *in ovo* and *in vitro*.

9. Isolation can take place either by the contraction of the vessels adjacent to the differentiating artery until the lumen is obliterated, or by the formation of a septum across the opening of the adjacent vessel into the artery.

10. The vitelline artery *in vitro* is differentiated from a tract of dilated vessels, and is large in calibre at isolation: no growth in diameter takes place in the next few hours of circulation. The vitelline artery *in ovo* is formed from less dilated vessels, and intervascular spaces are still seen within it after isolation. The whole artery then begins to grow in calibre, and becomes regular in outline.

11. The vessels to either side of the artery which are cut off from connection with it become venous in function. They form part of a plexus of vessels running dorsal to the vitelline artery, which returns blood to the heart from the hinder region of the area vasculosa. The formation of the plexus dorsal to the vitelline artery is preceded by a differentiation of isolated vessel spaces within the mesenchyme, which join up with the severed vessels on either side of the artery. When no circulation takes place, the isolated vessel spaces dorsal to the vitelline artery differentiate, but do not join up with other vessels.

12. *In vitro*, the layer of mesenchyme surrounding the vessels is much thicker than *in ovo*. This is due partly to the presence of a larger number of cells, but chiefly to a greater accumulation of intercellular fluid. The relations *in vitro* between the amount of intercellular space in the mesenchyme and the degree of dilation of the vessels show that when the vessels contract, fluid passes into the surrounding mesenchyme.

13. In the early pre-vascular mesenchyme, mitotic figures are very abun-

dant, and with the differentiation of the vessel network, the relative number of dividing cells steadily decreases. During the first few hours of circulation, mitosis is at a minimum throughout the vessel network, but as the vitelline artery differentiates, mitotic figures reappear in the wall. They become much more numerous in the artery *in ovo*, where a rapid growth in calibre takes place.

14. When vessels increase in length, this increase is not at first accompanied by endothelial cell division. The increase in circumference of the area vasculosa causes an increase in length of the individual meshes of the network, and not until the twenty-six-somite stage does compensatory mitosis in the general network take place.

15. When a vessel increases in length, and contracts in diameter, the endothelial nuclei elongate in the direction of the axis of the vessel. When the artery has differentiated and is increasing in diameter, many of the endothelial nuclei elongate, and are frequently orientated at right angles to the axis of the vessel.

16. There is evidence to show that elongation of a previously rounded nucleus may be caused by tensions acting within the cell. There is also evidence that when an elongated nucleus divides, the direction of the axis of the mitotic spindle tends to correspond with that of the elongation in the resting phase.

17. In virtue of this relation between tension, nuclear elongation, and mitosis a mechanism is suggested whereby the growth of an artery in its first stages of development would tend to decrease the resistance offered to the passage of the blood stream. At those points along the artery where the fluid friction generated by the circulation was greatest, excessive tensions would be set up within the wall, which would be manifested by nuclear elongation within the endothelium. When cell division took place, the directional increase in area of the endothelium under tension consequent on the orientation of the mitotic spindles would tend to relieve these tensions.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Photograph of a twenty-two-somite blastoderm, cultivated *in vitro*, and vitally injected. The injection was made into the left side of the blastoderm, and has caused the vessels in that area to contract, as is seen anterior to the head of the embryo. ×22.

Fig. 2. Photograph of the same blastoderm as that of fig. 1, eight hours later. The embryo now has twenty-eight somites, and the differentiation of the vitelline artery is progressing. ×22.

Fig. 3. Section through the right vitelline artery of a twenty-seven-somite blastoderm cultivated *in vitro*, to show the thickness of the mesenchyme layer dorsal to the artery, in which isolated vascular spaces such as (v_1) are to be found. The plane of section is indicated in text-fig. 16, a reconstruction of the whole vitelline artery area. ×180.

Fig. 4. Section through the right vitelline artery of a twenty-six-somite blastoderm *in ovo*, to compare with the corresponding artery *in vitro* of fig. 3. The mesenchyme layer above the artery is much more compact. ×190.

Fig. 5. Section through the vitelline artery area of a thirty-somite blastoderm, in which the posterior region was isolated and cultivated *in vitro*. The vessels of the dorsal venous plexus have dilated, while the vitelline artery has contracted. Much degeneration has taken place in the erythroblasts within the vessels, and in the surrounding mesenchyme, but not in the endothelium. ×300.

Fig. 6. Section through the area pellucida of a thirty-three-somite blastoderm *in ovo*, in the region of the vitelline artery, and parallel to the embryonic axis, to show the change in form of the vitelline artery from a flattened (fig. 4) to a rounded cross-section. The artery is the more ventral vessel, dorsal to which is the venous plexus. ×74.

PLATE II

Fig. 1. Photograph of a blastoderm of nine somites, cultivated *in vitro*. The vessels of the area vasculosa are not yet fully communicating. The position of the first somite relative to (*g*) in this, and the succeeding four photographs shows the forward migration of the somites. ×23.

Fig. 2. Photograph of the same blastoderm as that of fig. 1, 2 hours 20 minutes after fig. 1. The embryo now has ten somites. ×23.

Fig. 3. Photograph of the same blastoderm as that of fig. 1, 3 hours 35 minutes after fig. 1. The embryo now has eleven somites. The area vasculosa consists largely of fully differentiated vessels, which have been formed within the last hour by the fusion of separate vascular spaces. The vitelline artery area behind the somites consists of vessels of larger calibre than elsewhere. ×23.

Fig. 4. Photograph of the same blastoderm as that of fig. 1, 4 hours 30 minutes from fig. 1. The embryo now has twelve somites. Further differentiation of vessels has taken place. ×23.

Fig. 5. Photograph of the same blastoderm as that of fig. 1, taken 13 hours 50 minutes later. The embryo now has thirty somites. The circulation failed to take place at the appropriate time, and the vessels progressively dilated. The dark spots represent all that remains of the intervascular spaces. ×23.

Fig. 6. Photograph of blastoderm of eleven somites. The vessel network is differentiated caudally as far as the vitelline artery area. The three areas (*a*), (*b*) and (*c*) are drawn on a larger scale in text-figs. 1, 2 and 3 respectively. ×32.

PLATE III

Fig. 1. Photograph of the left vitelline artery area of an embryo of seventeen somites *in ovo*. The circulation has been in progress for one hour. ×37.

Fig. 2. Photograph of the same vitelline artery area as in fig. 1, 50 minutes later. The intervascular spaces have been outlined. ×37.

Fig. 3. Photograph of the same vitelline artery area as in fig. 1, 2 hours 10 minutes later. Isolation of the vitelline artery is beginning, and the vessels to either side of the future artery are contracting. ×37.

Fig. 4. Photograph of the same vitelline artery area as in fig. 1, 3 hours 10 minutes later. Isolation of the artery is more advanced on its cranial margin. ×37.

Fig. 5. Photograph of the same vitelline artery as in fig. 1, 5 hours 45 minutes later. The artery is now isolated on the caudal margin. ×37. The final stage reached by this area, 12 hours after the circulation had begun, is shown in text-fig. 19.

Fig. 6. Photograph of a blastoderm cultivated *in vitro* without the embryo, in which the vessels were made to contract by the injection of Indian ink. ×24. A drawing of a small area of this blastoderm under high magnification is shown in text-fig. 7.

Fig. 7. Photograph of a culture made by explanting a fragment of area opaca at the ten-somite stage. A network of vessels has been formed, which are shown up by their content of erythroblasts. ×20. The rectangle encloses the area drawn at higher magnification in text-fig. 26.

LIST OF ABBREVIATIONS

d.v.p. Dorsal venous plexus.
e.c. Extra-embryonic coelom.
g. Yolk granule on the surface of the blastoderm.
i.v.s.$_1$ Intervascular space in the vitelline artery area, small in size from its first differentiation.
i.v.s.$_2$ Intervascular space in the area opposite the middle somites, large in size at its first differentiation.
i.v.s.$_3$ Intervascular space in the area opposite the middle somites, small in size after the dilation of the vessels.
s_1 First somite.
s_4 Fourth somite.
v_1 Isolated vessel space above vitelline artery, identical with (v_1) in text-figs. 16 and 18.
v.a. Vitelline artery.

Fig. 1.

Fig. 2.

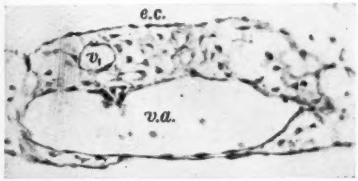
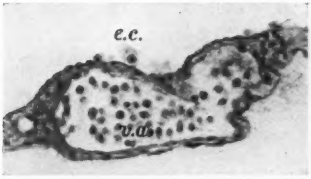


Fig. 3.



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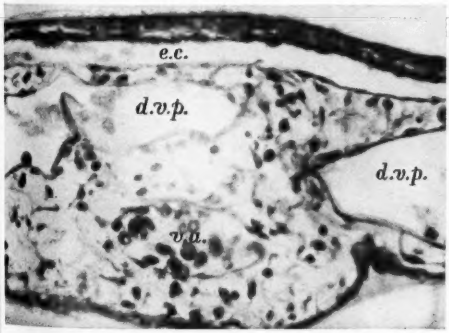

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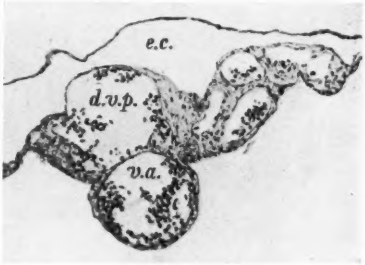

Fig. 6.

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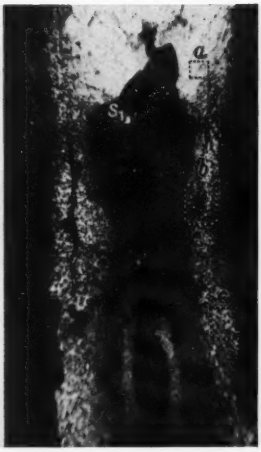

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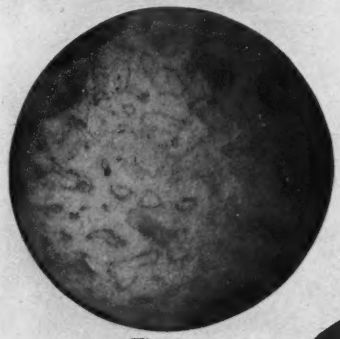

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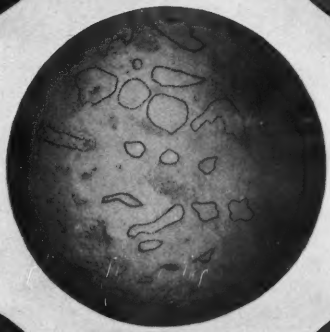

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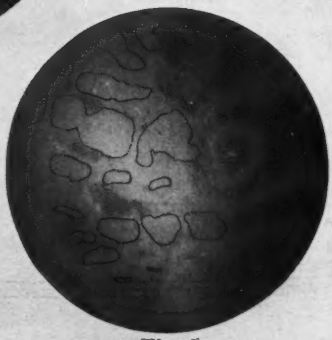

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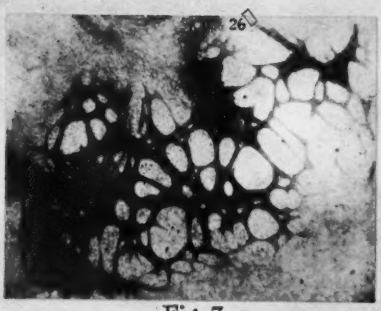

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THE SEX DIFFERENCE IN THE PROPORTION OF THE SUPRARENAL CORTEX OCCUPIED BY LIPOID IN GUINEA-PIGS OVER ONE YEAR OLD

By RAYMOND WHITEHEAD

From the Department of Pathology, Victoria University of Manchester

In a previous paper (Whitehead, 1934) it was shown that the proportion of the guinea-pig suprarenal cortex occupied by lipoid decreased progressively in both sexes from 14 to 168 days after birth, and that after 14 days the suprarenals from females contained relatively more cortical lipoid than those from males. In the present paper this sex difference is shown to be greater in guinea-pigs over one year old.

MATERIAL AND METHODS

The suprarenals of ten male and ten female guinea-pigs were studied. All the guinea-pigs were over one year old, but their exact ages were not known. The females had been used for breeding. The methods of examination were the same as those previously described (Whitehead, 1934). The proportion of the cortex occupied by lipoid was found by weighing drawings of Sudan III stained frozen sections. Since the weights vary directly with the areas of the drawings, the weight ratio (weight of the drawing of the lipoid-laden cortex)/(weight of the drawing of the total cortex) is also the area ratio (area of the lipoid-laden cortex)/(area of the total cortex), referred to as L/C.

RESULTS

The values of L/C are shown in Table I.

The darkly stained band in the cortex visible in Plate I, figs. 1–4, is the lipoid-laden cortex, of which the drawing was made for the calculation of L/C. Lipoid was present also in the following sites, but in such small quantity that it was neglected for the purposes of measurement: (1) the subcapsular rim of cortical cells, outside the lipoid-laden cortex, in all the glands; (2) an extremely small number of cortical cells situated between the inner border of the lipoid-laden cortex and the outer border of the medulla, in each of eight of the glands from males and in all those from females; and (3) cortical cells mingled with those of the medulla in thirty-six of the forty glands. The regular occurrence of lipoid in sites (1) and (3) was noted in younger guinea-pigs (Whitehead, 1934); lipoid was sought for in site (2) only in the present series, by an additional systematic examination. As in the younger guinea-pigs, all the sudanophil substances in all the glands contained cholesterol, demonstrated by the Schultz reaction.

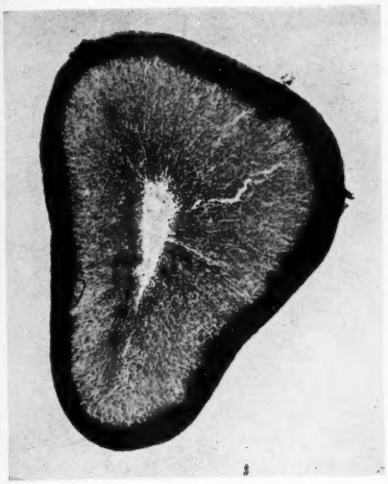
STATISTICAL TREATMENT OF RESULTS

The means of L/C for females exceed those for males. The significance of the differences between the means was determined by the t test, as used by Fisher (1932). In these and the similar tests described below, $n=18$. For left glands t was found to be 10·115, $P<0·01$; for right glands $t=9·725$, $P<0·01$. These results indicate that the odds against the occurrence of such differences as the result of chance exceed 100 to 1.

Table I. *Body weights of normal guinea-pigs over one year old and weights of drawings of the suprarenal cortex with the values of L/C thence obtained. Means in bottom row.*

	Left glands			Right glands		
	Weights (mg.) of drawings of cortex			Weights (mg.) of drawings of cortex		
Body weight (gm.)	Lipoid-laden	Total	L/C	Lipoid-laden	Total	L/C
			Males			
710	113	293	0·4	119	295	0·4
550	119	287	0·4	109	297	0·4
850	133	510	0·3	144	532	0·3
830	145	557	0·3	133	521	0·3
810	90	264	0·3	97	318	0·3
760	98	390	0·3	94	369	0·3
730	130	467	0·3	133	474	0·3
870	114	477	0·2	133	584	0·2
870	90	399	0·2	82	395	0·2
760	90	431	0·2	81	347	0·2
774	112·2	407·5	0·29	112·5	413·2	0·29
			Females			
500	310	400	0·8	261	350	0·7
650	187	285	0·7	200	289	0·7
640	294	425	0·7	294	426	0·7
620	183	272	0·7	217	332	0·7
620	168	235	0·7	184	271	0·7
640	182	312	0·6	173	291	0·6
620	151	247	0·6	133	228	0·6
610	194	311	0·6	208	333	0·6
580	137	246	0·6	138	288	0·5
780	175	365	0·5	165	340	0·5
626	198·1	309·8	0·65	197·3	314·8	0·63

The sex difference between the means of L/C might be due theoretically to a sex difference in the mean area of the total cortex alone, in the mean area of the lipoid-laden cortex alone, or in both of these quantities. The t test was therefore applied to each. The results of comparing the mean weights of the drawings of the total cortex were: for left glands $t=2·566$, $P=0·02$–$0·01$; for right glands $t=2·608$, $P=0·02$–$0·01$. The results of comparing the mean weights of the drawings of the lipoid-laden cortex were: for left glands $t=4·466$, $P<0·01$; for right glands $t=4·791$, $P<0·01$. The difference is thus significant in each of the four cases. The sex difference in the means of L/C is therefore due to sex differences in the mean areas of the total cortex and of the lipoid-



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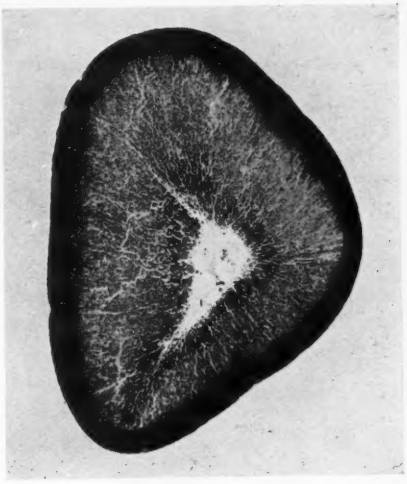

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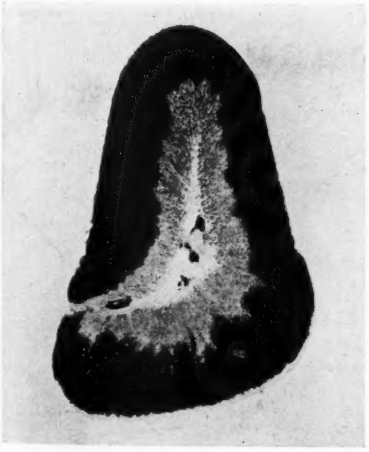

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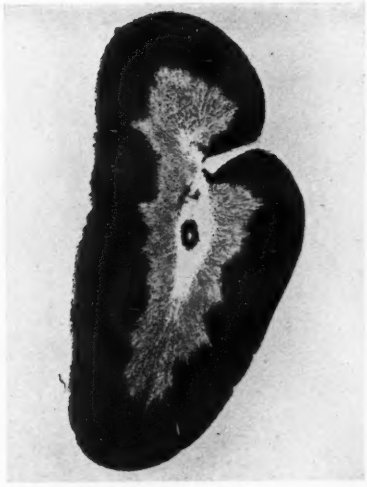

Fig. 4.

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laden cortex. The relative importance of the two quantities was determined by calculating, from data in Table I, the mean excess of the area of the total cortex, and the mean deficiency of the area of the lipoid-laden cortex, in males, expressed as percentages of the two respective mean areas for females. It was found that the mean area of the total cortex in males was 31·5 per cent. greater for left glands, and 31·3 per cent. greater for right glands, than in females. The mean area of the lipoid-laden cortex in males was 43·4 per cent. less for left glands, and 43·0 per cent. less for right glands, than in females. The smaller mean area of the lipoid-laden cortex in males is therefore the more important of the two factors producing the sex difference between the means of L/C in the guinea-pigs studied.

SUMMARY

1. The proportion of the cortex occupied by lipoid was determined for the suprarenals of ten male and ten female guinea-pigs over one year old.

2. The mean proportion of the cortex occupied by lipoid in males was 0·29 both for left and for right glands, in females 0·65 for left glands and 0·63 for right glands.

3. There was a statistically significant difference between the means for left glands from males and females, and between the means for right glands from males and females.

4. The mean area of the total cortex in males was 31·5 per cent. greater for left glands, and 31·3 per cent. greater for right glands, than in females.

5. The mean area of the lipoid-laden cortex in males was 43·4 per cent. less for left glands, and 43·0 per cent. less for right glands, than in females.

6. The histological results agree with those obtained on younger guinea-pigs.

I am indebted to Mr F. W. Priestley for calculating the statistics, and to Prof. S. L. Baker for criticism.

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Whitehead, R. (1934). *J. Anat.*, Lond., vol. lxix, p. 72.

EXPLANATION OF PLATE I

Figs. 1–4 show transverse frozen sections, stained with Sudan III and haemalum, of the suprarenals of normal guinea-pigs over one year old. All ×10. Lipoid appears black; lipoid-free cortex, grey; medulla, light grey or colourless. (Photographs by H. C. Taylor.)

Fig. 1. Left suprarenal from a male. Lipoid forms a narrow band at the periphery of the cortex.

Fig. 2. Right suprarenal from the same guinea-pig as fig. 1, showing the close resemblance between the two glands from one guinea-pig.

Fig. 3. Left suprarenal from a female. Lipoid forms a broad band in the outer part of the cortex. The black areas in the medulla represent groups of lipoid-laden cortical cells.

Fig. 4. Right suprarenal from the same guinea-pig as fig. 3.

A STUDY OF THE ADRENAL CORTEX OF THE RABBIT

By R. ROAF, B.A. (Oxon.)

From the Department of Zoology, the University, Liverpool

I. INTRODUCTION

Various authors have described a degeneration of the cells of the innermost zone of the adrenal cortex of the mouse. Masui and Tamura (1926) call this zone the reticular zone, and say that in the female it undergoes fatty degeneration at the beginning of pregnancy, and that after parturition there may be regeneration. Further they claim that there are changes in this zone which can be correlated with the oestrous cycle, but their grounds for this are obscure. Miller (1927) calls the innermost zone of the mouse adrenal cortex the X zone or transitory cortex. She says that the cells degenerate either fibrously or fattily during early maturity even if pregnancy does not occur, though pregnancy hastens the process. In her opinion the degeneration is an age phenomenon, and the zone is tentatively compared with the inner foetal zone of the human adrenal cortex—usually called the boundary zone.

Both Deanesley (1928) and Whitehead (1933) agree with Miller in all essential points. All these workers agree that:

(*a*) The zone which degenerates in the mouse is interlocked with the medulla.

(*b*) In the adult male this zone is not present, having degenerated fibrously at between 30 and 50 days of post-natal life.

(*c*) In the castrated male the zone persists into adult life but eventually degenerates, usually at about 90 days.

(*d*) In the virgin female, degeneration is much slower than in the normal male and the zone usually persists throughout a considerable part of adult life.

Deanesley (1928) and Miller (1927) further show that, in the pregnant female, the degenerative process is hastened, the zone disappearing completely before parturition.

During the course of the present investigation Waring (1935) has shown that the interlocking or X zone in the mouse adrenal is the remains of the cortical anlage, and as such is probably homologous with the boundary zone of Man.

A study of the adrenal cortex of the rabbit was undertaken to see whether any changes occur there comparable with those found in the mouse.

II. MATERIAL AND TECHNIQUE

Rabbits of both sexes and of widely different ages were used. The animals were chloroformed, and the organs were dissected out while the body was still warm. In most cases the adrenals, gonads and uterus were removed. Portions of the liver and spleen were also removed from later animals. The organs were generally fixed in Bouin's fluid, this being followed by the usual process of dehydrating and embedding in wax. Either Ehrlich's haematoxylin or methylene blue and eosin were used to stain the sections.

In order to test for fatty substances, a few adrenals and ovaries were fixed in 5 per cent. formalin, washed, placed in gum arabic, frozen, cut, and stained with Sudan III. There were large globules in the inner part of the cortex and in the corpus luteum, which gave the typical staining reactions of "fats," some staining red, others orange. Also it is well known, from chemical evidence, that the adrenals and ovaries contain large amounts of cholesterol (either free or combined). For these reasons, therefore, it has been concluded that the vacuoles in paraffin sections represent globules of some lipoid such as cholesterol. [W. Shaw (1925) concluded that vacuoles in the cells of the corpus luteum, seen in paraffin sections, represented fatty material. Cooper (1925) came to the same conclusion about vacuoles in the human adrenal cut into paraffin sections.]

III. OBSERVATIONS

(*a*) *Adrenal cortex of adult rabbit*

From an examination of the adrenal glands of sixty rabbits (and twenty-four embryos) of very different ages and of both sexes it is clear that there is not at any time a clearly marked sex difference such as occurs in the mouse.

In the adrenal gland of an adult rabbit of either sex four cortical zones can be distinguished (see Plate I, fig. 1).

(*a*) A narrow peripheral zone in which the cells are arranged in arches, the glomerular zone (*g.z.*).

(*b*) A wider zone in which the cells are arranged in radial columns, the zona fasciculata (*f.z.*).

(*c*) A zone of variable width, whose cells are not arranged in any particular order. It is definitely not fasciculate and is here called reticular, in accordance with popular usage, though this term does not always describe the arrangement well (*r.z.*).

(*d*) A variable amount of darkly staining cells with faint or unrecognisable intercellular boundaries. The cells are arranged in groups of three or four both within and around the medulla (*m.*) with which interlocking occurs. This tissue will be referred to throughout the present paper as the interlocking zone (*i.z.*).

Mitsukuri's description of the adrenal of the rabbit (7) makes no mention of the innermost cortical region ((*d*) above), neither is it shown in his figure.

In order to get some clue to the identity of the various cortical regions, a brief outline of the development of the gland has been worked out.

(*b*) *Development*

No attempt has been made to work out the development in detail. Only a very incomplete series of embryos and young animals has been obtained, and from these a mere outline of the development has been suggested.

In a 22-day-old embryo (6 cm. long) the cortex and medulla cannot be distinguished as tissues, i.e. their respective cells, though clearly recognisable, are indiscriminately mixed.

At 26 days (embryos approximately 9 cm. long) the zona glomerulosa of the cortex has differentiated as a peripheral layer, but the rest of the cortex is undifferentiated and is still mixed with the medulla.

In embryos of 28 days and in young animals, 1 day after birth, the separation of cortical from medullary tissues has gone further, and the process of differentiation of the cortex seems to be proceeding from without inwards.

At 2 days after birth the following zones can be distinguished:

(*a*) A narrow peripheral zona glomerulosa.

(*b*) A somewhat wider zona fasciculata.

(*c*) A zone of about the same thickness as the fasciculate and of similar cells. These, however, are not arranged in any particular order and the tissue is not yet either fasciculate or reticular.

(*d*) A relatively small amount of darkly staining cells, arranged in groups of three or four, both inside and around the medulla with which interlocking occurs. These cells, comprising the interlocking zone, appear to have been left behind when the cortex separated from the medulla.

In animals of 8 days and of 3 weeks old the condition is substantially the same, there still being no true reticular zone and comparatively little interlocking zone. It is not till 9½ weeks after birth that the tissue lying between the fasciculate zone and the interlocking zone can be recognised as reticular, and even at this age the reticular zone is not always clearly differentiated. When it is present it is narrow. The interlocking zone is still small at 9½ weeks.

At 11½ weeks old the reticular zone is larger and more clearly differentiated. Both this zone and the interlocking zone increase as maturity is reached till, in an occasional gland, the reticular zone may come to occupy half the total thickness of the cortex, and the interlocking zone, still mixed with the medulla, may exceed this latter tissue in area in cross section.

No difference between the adrenals of the two sexes can be distinguished in sub-adults (age and sex being confirmed by examination of the gonads).

As already stated, in the adrenal of the adult, the arrangement is substantially the same in males and females. Nevertheless, both the reticular and interlocking zones tend to be smaller on an average in males. (Maturity was confirmed by fertility and by histological study of the gonads.)

Thus from these observations it looks as though the innermost interlocking zone of the adrenal cortex of the rabbit represents the remains of the original foetal cortex, after reticular, fasciculate and glomerular zones have become differentiated.

Table I.

No. of animal	Sex	Time of year	State of animal	Follicles	Corpora lutea	Uterus	Extent of interlocking zone	Reticular zone		
								Width	Arrangement of cells	Fatty degeneration
2	♀	December, 1932	Mature, non-pregnant	Ripe	Fatty degenerating	Follicular	Little	Narrow	Close	Small fat vacuoles in some cells
5	♀	,,	Mature, unmated	Ripe	None	Small atrophic	Little	Wide	Open	None
6	♀	,,	Mature, non-pregnant	½ Grown small	Old—still some fat vacuoles	Prefollicular	Little	Wide	Open	Large extracellular fat vacuoles
7	♀	,,	Mature, pseudo-pregnant or true pregnant	½ Grown small	(1) Nearly mature (2) Late—large fat vacuoles	Luteal	Little	Wide	Open	Large extracellular vacuoles
8	♀	,,	Pregnant, 3 cm. embryos	—	—	—	None	Moderate	Open	None
9	♀	,,	Pseudo-pregnant or true pregnant	½ Grown	(1) ½ Grown (2) Late—large fat vacuoles	Half-way to luteal	Moderate	Wide	Open	Small extracellular vacuoles
10	♀	,,	Pseudo-pregnant	½ Grown	½ Grown	Luteal	Very little	Wide	Open	None
11	♀	,,	Pregnant, 9 cm. embryos	Ripe	Mature	—	Moderate	Moderate	Open	Very small vacuoles in a few cells
12	♀	,,	Pregnant, 5·25 cm. embryos	—	—	—	Plentiful	Wide	Open	None
13	♀	March, 1933	Old, 3 weeks after parturition, lactating	Ripe	Late—some small fat vacuoles	—	Plentiful	Narrow	Close	Small intracellular vacuoles
17	♀	,,	Mature, unmated, 18½ weeks old	Ripe	None	—	Moderate	Narrow	Open	None
29	♀	,,	8 days after parturition, lactating	½ Grown	(1) ½ Grown (2) Old—fat vacuoles	—	Plentiful	Narrow	Slightly open	Large intracellular and small extracellular vacuoles
30	♀	April, 1933	Old, 2 days after parturition, lactating	Ripe	(1) Old—fat vacuoles (2) Corpora albicantia	Follicular	Plentiful	Moderate	Open	Extracellular vacuoles
39	♀	,,	5 days after parturition, non-lactating	Ripe	Old—small fat vacuoles	Follicular	Plentiful	Moderate	Close	Large intracellular fat vacuoles
40	♀	,,	Never been pregnant before; mated for the first time March 6th—pseudo-pregnant; remated March 24th—22 days pregnant when killed	½ Grown	(1) Nearly mature, i.e. 22 days old (2) Old—some fat vacuoles, i.e. 40 days old	Luteal	Moderate	Moderate	Open	Vacuoles breaking down to form intercellular spaces
46	♀	June, 1933	Pseudo-pregnant or true pregnant	¾ Mature	(1) Very early (2) Old—fat vacuoles	Nearly luteal	Very little	Moderate	Half open	Intracellular fat vacuoles
47	♀	,,	6 days after parturition, lactating	½ Grown	Old degenerating, few fat vacuoles	Half-way to luteal	Moderate	Moderate	Half open	Very few extracellular fat vacuoles
48	♀	October, 1932	Pregnant, 2·8 cm. embryos	—	—	—	Plentiful	Thin	Close	None
49	♀	,,	Pregnant, 3·6 cm. embryos	—	—	—	Plentiful	Thin	Close	None
50	♀	,,	Pregnant, 9 cm. embryos	—	—	—	Moderate	Thin	Half open	None
51	♀	,,	Pregnant, 9·4 cm. embryos	—	—	—	Plentiful	Wide	Open	Many small intracellular vacuoles

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Table I.

No. of animal	Sex	Time of year	State of animal	Width of interlocking zone	Uterus	Follicles	Corpora lutea
2	♀	December, 1832	Mature, non-pregnant	Little	Small	Ripe	Regenerating
5	♀	"	Mature, unmated	Little	Small	Ripe	None
6	♀	"	Mature, non-pregnant	Little	Grown	Prefollicular	Old
7	♀	"	Mature, pseudo-pregnant or true pregnant	"	Grown	small	Old
						(1) fatty	
						(2) large fat vacuoles	
8	♀	"	Pregnant, 3 cm. embryos	—	—	—	—
9	♀	"	Pseudo-pregnant or true pregnant	"	Grown	(1)	
						(2)	
10	♀	"	Pseudo-pregnant	Very little	Grown	½ Grown	Old
11	♀	"	Pregnant, 3 cm. embryos	—	—	—	—
12	♀	"	Pregnant, 2·2 cm. embryos	—	—	—	—
13	♀	March, 1833	Old, 3 weeks after parturition, lactating	—	Ripe	fat	Large
17	♀	"	Mature, unmated, 18½ weeks old	—	Ripe	—	None
20	♀	"	8 days after parturition, lactating	—	½ Grown	(1)	
						(2) fat vacuoles	
30	♀	April, 1833	Old, 2 days after parturition, lactating	—	Ripe	(1) fat vacuoles	Follicular
						(2) area albicantia	
39	♀	"	8 days after parturition, not lactating	—	Ripe	—	Old
40	♀	"	Never been pregnant; mated for the first time March 6th—pseudo-pregnant; remated March 24th—22 days pregnant when killed	—	½ Grown	(1)	
						(2)	
		June, 1833	Pseudo-pregnant or true pregnant			½	Mature
						(1)	
						(2)	
		"	3 days after parturition, lactating		Grown	½	Old
							fat
		October, 1832	Pregnant, 2·2 cm. embryos	—	—	—	—
		"	Pregnant, 3·0 cm. embryos	—	—	—	—
		"	Pregnant, 3 cm. embryos	—	—	—	—
		"	Pregnant, 3·4 cm. embryos	—	—	—	—

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(*c*) *Does the interlocking zone degenerate?*

All the facts relevant to the present investigation are given for adult animals in Tables I and II. From an examination of the data, it is fairly clear that the X or interlocking zone does not usually undergo degeneration in either sex, but persists well into adult life. As no animals were obtained of known advanced age, its ultimate fate is uncertain. However, apart from female 8, which is not understood, all the adults examined, male and female, pregnant and non-pregnant, showed the interlocking zone well developed without any signs of degeneration. In this the rabbit is strikingly different from the mouse.

Table II

No. of animal	Date of castration	Date of death	Interlocking zone	Reticular zone: Width	Reticular zone: Arrangement of cells	Reticular zone: Fatty vacuolisation
1	Not castrated	December, 1932	Little	Very narrow	Compact	None
3	,,	,,	Moderate	Moderate	Open	None
4	,,	,,	Plentiful	Moderate	Open	None
45	,,	June, 1933	Very little	Narrow	Open	None
58	,,	September, 1933	Moderate	Moderate	Half open	Many intracellular and a few extracellular vacuoles
60	,,	December, 1933	Plentiful	Moderate	Half open	None
62	,,	,,	Plentiful	Narrow	Close	None
64	,,	,,	Moderate	Narrow	Close	None
44	March 24th, 1933	June 24th, 1933	Moderate	Moderate	Open	None
52	July 31st, 1933	September 22nd, 1933	Little	Narrow	Close	Small intracellular vacuoles
53	,,	,,	Little	Moderate	Close	Small intracellular vacuoles
54	,,	September 30th, 1933	Little to moderate	Narrow	Close	Many intracellular vacuoles
55	,,	September 28th, 1933	Moderate	Wide	Half open	Many extracellular vacuoles
56	,,	,,	Little to moderate	Narrow	Half open	Both extracellular and intracellular vacuoles
57	,,	September 30th, 1933	Little	Moderate	Close	Many intracellular vacuoles
58	October 6th, 1933	December 8th, 1933	Little	Moderate	Mainly close, one small open area	None
61	,,	,,	Plentiful	Moderate	Close	Very few vacuoles
63	,,	December 28th, 1933	Moderate	Moderate	Open	Very few vacuoles

(*d*) *Fatty vacuolisation of the reticular zone*

In the adrenal glands of adult males and females the reticular zone often shows considerable vacuolisation, due, apparently, to the removal of fatty substances (see p. 127 above). This fatty vacuolisation is found more often in females than in males. It has not been observed at all in sub-adults.

Where there is no obvious vacuolisation, the cytoplasm has a very even, finely reticular or alveolar appearance, as shown in Plate I, fig. 2. When fatty vacuolisation is present, the degree differs greatly in different animals, and from

the series of glands observed the process of vacuolisation appears to be as follows. In the first place small vacuoles appear scattered irregularly throughout the cytoplasm (see Plate I, fig. 3). These vacuoles become larger and coalesce (Plate I, fig. 4) until practically the whole cytoplasm is replaced by vacuoles. Finally such cells degenerate leaving large intercellular vacuoles (Plate I, fig. 5, *i.v.*), which in their turn ultimately disappear leaving large irregular spaces (Plate I, fig. 5, *i.s.*). There is no evidence that this process ever results in the destruction of the whole zone.

(*e*) *Correlation between fatty vacuolisation of the reticular zone and of the corpora lutea*

Fatty vacuolisation, very similar to that described above, is found also in the corpus luteum of the ovary (cf. also Deanesley, 1930). The main difference is that, in the final stage, the undegenerated cells are packed closely together in the corpus luteum to form a corpus albicans, whereas in the reticular zone they form a very loose reticulum. Exactly the same stages of fatty vacuolisation can, however, be traced in both structures.

From a histological study of the adrenals and gonads of fifteen adult females, the interesting fact has come to light that the degree of fatty vacuolisation in the reticular zone is very closely correlated with the degree of the same process in some of the corpora lutea of the ovary. The correlation is not always with the most recently formed corpora lutea, but where there are corpora lutea in the ovary showing any degree of vacuolisation, that degree is correlated with the degree of the same phenomenon in the reticular zone of the adrenal cortex. Where there is no vacuolisation in any of the corpora lutea, there is none in the reticular zone. Not more than one degree of vacuolisation has been found in any one ovary.

When the females are arranged according to increasing amount of fatty vacuolisation of the reticular zone, they fall into the following series:

Little or no fat	Intracellular fat	Extracellular fat
5, 17, 10, 11	13, 47, 2, 39, 46	29, 9, 30, 6, 7, 40

When these same animals are arranged according to the increasing amount of fatty vacuolisation of their corpora lutea, they fall into the following series:

Little or no fat	Intracellular fat	Extracellular fat
5, 17, 10, 11	13, 39, 2, 46, 47	29, 30, 9, 7, 6, 40

Although the two series are not exactly the same, and although the numbers are small, it is clear that there is a very close correlation between the degree of fatty vacuolisation of the reticular zone of the adrenal cortex and that of the corpus luteum.

Any phenomenon which is correlated with the development of the corpus luteum might be expected to show correlation with pregnancy. When the rabbit material is examined with this in view, it is seen that, in about two-thirds of

the total females used, the degree of fatty vacuolisation is correlated with the length of time pregnancy has lasted.

More animals are available for a consideration of pregnancy than are available in considering the condition of the corpora lutea. The ovaries of 8, 48, 49, 12, 50 and 51 are unknown. Table III includes all the adult females used, and from an examination of this table it is seen that the following

Table III

Animals in order of increasing degrees of fatty vacuolisation of the reticular zone		Facts relevant to pregnancy
Little or no fat	5	Unmated
	17	Unmated
	10	Early pseudo- or true pregnant
	8	With 3 cm. embryos
	48	With 2·8 cm. embryos
	49	With 3·6 cm. embryos
	12	With 5·25 cm. embryos
	50	With 9 cm. embryos
	11	With 9 cm. embryos
Intracellular fat	13	21 days after parturition, lactating
	47	6 days after parturition, lactating
	51	With 9·4 cm. embryos
	2	Non-pregnant
	39	5 days after parturition, non-lactating
	46	Early pseudo- or true pregnant
Extracellular fat	29	8 days after parturition, lactating
	9	Early pseudo- or true pregnant
	30	2 days after parturition, lactating
	6	Non-pregnant
	7	Early pseudo- or true pregnant
	40	22 days pregnant. 6 cm. embryos

animals, arranged according to increasing degrees of fatty vacuolisation of the reticular zone, at the same time fall into order in regard to the length of time that has elapsed since fertilisation:

5, 17, 10, 8, 48, 49, 12, 50, 11, 51, 39, 29.

Females 30 and 47 show a less exact correlation but are not entirely out of series.

In the remaining females 13, 2, 46, 9, 6, 7 and 40, however, the degree of fatty vacuolisation of the reticular zone obviously cannot be correlated with pregnancy. Of these, 2, 46, 9, 6 and 7 are all non-pregnant, pseudo-pregnant or early pregnant, yet all show a fairly advanced stage of fatty vacuolisation of the reticular zone; and female 40, which is only about three-quarters through pregnancy, shows a very advanced stage. Female 13, on the other hand, is late post-partum (lactating), yet it has comparatively little vacuolisation of the reticular zone.

Clearly, therefore, fatty vacuolisation of the reticular zone is not very closely correlated with pregnancy.

When only those animals are considered for which facts are available in regard to both pregnancy and condition of the ovaries, it is very clear that the

real correlation is between reticular zone and corpora lutea, not between reticular zone and state of pregnancy or post-pregnancy (see Table IV).

In females 46, 9, 7, 40 and 29 (see Table I) there are two sets of corpora lutea in the ovaries, one set young, due to more recent matings, and another set of older ones. It is the latter which show stages of fatty vacuolisation comparable with the stages found in the reticular zone of the adrenal. These particular animals show very clearly that correlation between the adrenal cortex and the sexual phase would not be obtained if the state of the uterus were taken as a criterion: in my series the endometrium is correlated with the younger series of corpora lutea, while the adrenal cortex is correlated with the older series wherever two series of corpora lutea are present.

Table IV

A		B		C
Females arranged in order of increasing vacuolisation of corpus luteum		Females arranged in order of increasing vacuolisation of reticular zone		Facts relevant to pregnancy for females in column B
Little or no fat	5	Little or no fat	5	Unmated
	17		17	Unmated
	10		10	Pseudo-pregnant or very early pregnant
	11		11	With 9 cm. embryos
Intracellular fat	13	Intracellular fat	13	21 days after parturition, lactating
	39		47	6 days after parturition, lactating
	2		2	Non-pregnant
	46		39	5 days after parturition, non-lactating
	47		46	Pseudo-pregnant or very early pregnant
Extracellular fat	29	Extracellular fat	29	8 days after parturition, lactating
	30		9	Pseudo-pregnant or very early pregnant
	9		30	2 days after parturition
	7		6	Non-pregnant
	6		7	Pseudo-pregnant or very early pregnant
	40		40	22 days pregnant—with 6 cm. embryos

Such vacuolisation of the reticular zone, if repeated with every set of corpora lutea, should result in the progressive destruction of the zone, unless there is regeneration of tissue after each period of degeneration. There is, at present, no evidence either for progressive degeneration or for regeneration: the matter needs further investigation.

(*f*) *Fatty vacuolisation of the reticular zone of the male*

Fatty vacuolisation is found in the male gland, but very few observations have been made and the condition is not understood (see Table II).

Of eight normal males, killed at different times of the year, the one killed in September alone showed appreciable fatty vacuolisation; the remaining seven had none.

Of ten castrated males, killed at different times of the year, the six killed in September had excessive fatty vacuolisation of the reticular zone, two of the others had a very small amount and the remaining two had none. In all these cases the interval between castration and removal of the adrenal glands was between 2 and 3 months.

There is no evidence that castration has any effect on the adrenal cortex, but the numbers are too small to justify any definite statement.

The fact that fatty vacuolisation was found in all the males killed in September and scarcely at all in those killed at other times of the year suggests the possibility of seasonal variation, but the evidence is too slight to justify any conclusion.

IV. DISCUSSION AND SUMMARY

From the observations recorded above it is clear that, in the adult rabbit of either sex, there are four zones in the adrenal cortex.

The innermost cortical zone is interlocked with the medulla, and from the available embryological evidence it appears to represent the remains of the foetal cortex after the reticular, fasciculate and glomerular zones have been differentiated. As such it would be homologous with the X zone of the mouse adrenal.

The interlocking zone does not appear to undergo degeneration, fatty or fibrous, either with advancing age or with pregnancy, and in this the rabbit differs greatly from the mouse. (Its absence in female 8 is not understood.)

Fatty vacuolisation has been found in the adrenal cortex of the rabbit, but it occurs in the reticular zone, not in the interlocking zone. Here again the rabbit is strikingly different from the mouse.

The fatty vacuolisation of the reticular zone does cause the breakdown of some cells, but there is no evidence that it ever causes degeneration of the whole zone.

The degree of fatty vacuolisation of the reticular zone is closely correlated with the degree of similar vacuolisation of certain corpora lutea in the ovaries. As this correlation is not always with the most recently formed corpora lutea, it is clear that there cannot be any close correlation with pregnancy or with any other phase of the sexual cycle as such. The significance of the observed correlation is not understood.

The possibility presents itself that this correlation is not specific, but that both reticular zone and corpora lutea may be involved in a generalised movement of fatty substances throughout the body. This is improbable. For instance Elliott (1914) found that in cases where the general body fat was exhausted, that of both the corpora lutea and the adrenals was still retained. Also, during the present investigation, an examination of thirteen males showed no suggestion of correlation between the liver and adrenal in regard to fat content. It seems probable, therefore, that the observed correlation between fatty vacuolisation of the corpora lutea and of the reticular zone is specific, and is not part of a generalised condition.

It is not known whether the fatty vacuolisation of the reticular zone of the rabbit adrenal is in any way related to the fatty degeneration which is often found in the interlocking or X zone of the mouse adrenal. At present the phenomena appear to be unrelated.

I wish to thank Mrs R. C. Bisbee for much invaluable help and for performing the castrations.

I also wish to thank Mr H. Waring for the illustrations.

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EXPLANATION OF PLATE I

Fig. 1. Sector of T.S. of adrenal of adult rabbit. Bouin, haematoxylin and eosin. ×75.

Fig. 2. Cells of zona reticularis of rabbit adrenal, without fatty vacuolisation. Bouin, haematoxylin and eosin. × approx. 300.

Fig. 3. Cells of zona reticularis of rabbit adrenal, with small intracellular vacuoles. Bouin, haematoxylin and eosin. × approx. 300.

Fig. 4. Cells of zona reticularis of rabbit adrenal, with large intracellular vacuoles. Bouin, haematoxylin and eosin. × approx. 300.

Fig. 5. Cells of zona reticularis of rabbit adrenal, with large extracellular vacuole and with some cellular degeneration giving irregular intercellular spaces. Bouin, haematoxylin and eosin. ×300.

Fig. 1.

Fig. 2.

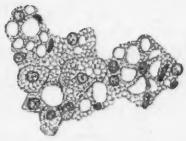


Fig. 3.

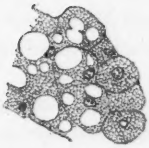


Fig. 4.

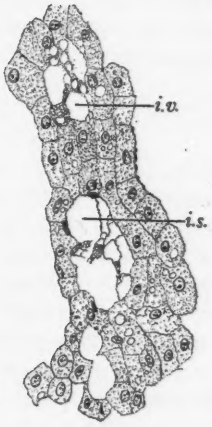


Fig. 5.

UNIV. OF MICH.

A DETAILED STUDY OF MOVEMENT OF THE WRIST JOINT

By R. DOUGLAS WRIGHT, M.S.

INTRODUCTION

The most precise and the most differentiated movements of the limbs are those which occur in the hand. It is generally assumed that this refinement is based on the mobility of the thumb and the lengthening of the digits associated with a highly developed neuromuscular mechanism. On closer study there appears a much more stable basis for this free but accurate movement. The range of mobility of the wrist joint is as great as that of the shoulder joint, but added to this range of mobility there is a constant steadiness resulting from the intricate admixture of arthrodial, enarthrodial, and ginglymus types of articulation.

The descriptions which are given in text-books of the actual movements of the carpus are very abbreviated, and in most cases inaccurate. Apart from the anatomical exercise of understanding the changes in the axes of the short bones taking part in the most complicated joint in the body, the movements of this joint in the use of the hand and the increasingly accurate recognition of conditions of disease and trauma in the carpal bones and associated joints render a precise knowledge of movements of these essential to accurate clinical diagnosis, treatment and prognosis.

METHOD OF INVESTIGATION

The kinematics of the wrist joint have been studied by Fick (1), by inspection of the moving dead joint and plane X-ray photographs; by Bryce (2) using plane X-ray photographs of the living hand; by Johnston (3) who fixed the dead joint in varying positions and then dissected the bones, and by van Bonin (4) who investigated the movements of a living hand by stereoscopic X-ray films. Most of what is recorded in the present report will be found in those papers, but as these results are widely different from many of the previous statements, the observations on mechanism will be set out without reference to the previous articles. It is an intricate subject for description, and reference to other similar or conflicting observations is confusing.

The following methods of investigation were employed:

(1) Stereoscopic X-ray films of the movements of the living hand were taken.

(2) The implantation of long needles into the several bones of the forearm, of the proximal and of the distal carpal rows, allowed an observation of the

axial movement of the bones in a recently dead hand when the wrist was motivated by pulling on appropriate tendons and when the ligaments were still intact.

(3) As a check on these observations and to aid visualisation of the movements represented in the two preceding methods, wrist joints, fixed but lax, were dissected and arranged to correspond to the disposition of the living bones seen stereoscopically and the dead ones "seen" by the movements of the needles. This also promoted renewed observation on the anatomy of the joints and joint surfaces.

ANATOMICAL CONSIDERATIONS

The first anatomical feature studied was that of the curves of bones, collectively and separately. The proximal surfaces of the proximal row of carpal bones, when viewed by an antero-posterior röntgenogram, form an almost perfect arc of a circle of which the centre is at the middle of the waist of the capitate bone (Plate I, fig. 1). Secondly when the navicular has its long axis parallel to that of the radius, the ulnar surface forms with the distal surface of the lunate bone about one-third of the circumference of a circle, the centre of which is approximately the centre of the head of the capitate (Plate I, fig. 3). This is necessarily so because it is the curved radial surface of the capitate which fits into this line. The antero-posterior curve on the distal capitellum of the navicular has its axis in line with the axis of the antero-posterior curve of the head of the capitate, i.e. a line passing transversely in the coronal plane through the centre of the head of this bone.

The ligaments of the wrist joint are most strongly developed on the volar aspect. The transverse ligament of the carpus needs no detailed mention; its function in maintaining the transverse arch of the carpus, in addition to that of a retinaculum, is not always appreciated. The strong transverse ligament which joins the volar surface of the triquetrum to that of the navicular is concerned with two movements which will be detailed later; it forms the volar surface of the cup in which the head of the capitate moves in the movements of extension, and when the hand is in the straight position with this ligament relaxed, ulnar deviation now produces a widening of the angle between the long axes of the triquetrum and the navicular in the coronal plane. This allows the greater width of the distal part of the hamate to shift proximally in this movement. The two collateral ligaments, between attachments, have axes in different lines, the ulnar ligament is distributed to the ulnar, volar and dorsal surfaces of the triquetrum and hamate; the longitudinal portion of the radial collateral ligament is attached wholly to the volar surface of the styloid process of the radius and to the volar surface of the tubercle of the navicular; this ligament prevents the movement of the navicular past the straight position in dorsi-flexion, i.e. extension, of the wrist.

The inter-osseous ligaments of the two carpal rows vary greatly in strength. That uniting the lunate to the navicular is very poorly developed in the middle portion; the volar and dorsal portions are much longer than the distance

between two bones when they are approximated, this allows the movement of the navicular bone relative to the lunate which occurs in flexion and that of the lunate on the navicular in extension. The relative immobility of the triquetrum on the lunate is due more to the large surface of apposition and the volar and dorsal ligaments than to the inter-osseous ligament. The ~~lunate~~ hamate and capitate bones are firmly united by the massive inter-osseous ligament; the capitate is very feebly joined to the lesser multangular and the greater multangular is weakly joined to this. The freedom of the multangulars will be noticed when the description of the deviations of the hand in a coronal plane are described.

Synovial membranes of the wrist joint are mainly studied and reported relative to complexity; this may account for occasional localisation of disease to isolated portions of the membrane. The most noticeable feature of the membranes of the radiocarpal and transverse carpal articulations is the extent of them; when the joint is in the resting position they present as extensive pouching folds especially on the dorsum in the radiocarpal joint and on the volar surface of the transverse articulation. Associated with these more marked folds synovial fringes frequently occur.

The facets which appear on the articular cartilages of the bones are usually the result of continuous contact with an opposing facet, but some of those occurring in the wrist joint are the result of occasional contact, e.g. that between the hamate and lunate, that between the neck of the capitate and the anterior edge of the waist of the navicular.

MOVEMENTS

The changes in arrangement of the carpal bones from the disposition which is present when the hand is in the straight position will be described in ulnar deviation, radial deviation, flexion, extension and forced extension. There are some features of the normal straight position which must be detailed before proceeding with this description (Plate I, figs. 1 and 2).

The distal surface of the lunate faces distally and slightly dorsally, the triquetrum is in a straight line with the bones of the forearm, but the navicular lies with its long axis at 30° anteriorly to the coronal plane of the forearm; it is slightly rotated round this long axis so that the anterior portion of the distal extremity comes to lie in front of the head of the capitate. The greater and lesser multangulars ride on the middle portion of the distal capitellum of the navicular. The capitate and the hamate lie in line with the metacarpals, the former inclined slightly dorsally relative to the lunate; the hamate has its axis parallel to that of the capitate. Even when the hand is maintained in the straight position, the position of the radial carpals may be modified by movements of the thumb. In extension of this digit the greater multangular moves radially to the lesser multangular, and in flexion it rotates on the head of the navicular; a portion of it is then in front of the lesser multangular in the antero-posterior view. In abduction of the thumb the slight movement of the greater

multangular is accentuated by a 10° increase in the volvar flexion of the navicular.

Ulnar deviation (Plate I, fig. 3) is mainly a gliding movement of the proximal row of the carpal bones on the radial and triangular ligament surfaces. The centre of this movement is the centre of the waist of the capitate, the line of movement is a portion of the arc formed by the proximal surfaces of the proximal carpal row. The ulnar edge of the lunate comes to lie slightly radial to the ulnar border of the radius.

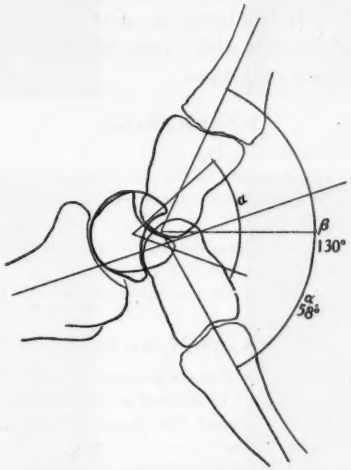
The extent of this movement is about 26°, but the total deviation of the middle line of the capitate and third metacarpal from the sagittal plane of the supinated forearm is about 32° in the coronal plane. This discrepancy of 6° is found in the movement of the distal carpal row on the proximal row. The axis of this movement is antero-posterior through the centre of the head of the capitate; the axis of the capitate moves in a coronal plane through 6°; the hamate, because it is closely bound to the capitate, moves proximally toward and often makes contact with the lunate. The wedge-like action of the hamate splays the proximal row slightly, as previously noticed. The greater and lesser multangulars follow the capitate, and the long axis of the navicular swings into longitudinal axis of the forearm with the multangulars on the most volar portion of the cartilage of the navicular capitellum and moved approximately 5 mm. in the ulnar direction relative to this surface.

Throughout this report, the extent of a movement will be referred to as the angle between two planes, two lines or that between a plane and a line in a given plane. The extent of movement at the wrist joint is usually given as so many degrees, but no specified lines are given. The angle recorded between the hand and forearm will vary greatly according to whether the radial or styloid process or the articular surface of the radius is taken as the point of angle and what are the distal points of the line; ranges of movement reported without specification of points of reference are therefore valueless.

Radial deviation (Plate I, fig. 4) in the coronal plane is possible to the extent of 22° between the middle line of the third metacarpal and the sagittal plane of the forearm. This movement takes place at the transverse carpal row entirely; no movement of the proximal row as a whole occurs relative to the radius. The axis of the movement is antero-posterior through the centre of the head of the capitate; the hamate is carried distally away from the lunate and allows the triquetrum to more closely approach the capitate: the multangulars move 3–5 mm. radially and dorsally on to the most proximal portion of the facet on the capitellum of the navicular. This bone becomes slightly more volar-flexed and rotated on the long axis than is the case in the resting position. This volar movement of the navicular and the distal movement of the hamate produce about 8 mm. of narrowing of the distance between the tuberosity of the navicular and the lateral border of the triquetrum.

Flexion of the wrist (Plate I, fig. 5, and text-fig. 1) is compounded of movement at the radiocarpal and at the intercarpal joints. The lunate and

triquetrum flex 30°, so that the distal surface of the lunate faces distally and slightly volarly. The navicular swings still further volarly on the lunate, so that the proximal surface points almost directly dorsally and the long axis of the bone in the sagittal plane is at right angles to the long axis of the forearm. The distal facet on the proximal portion of the navicular thus forms the dorsum of the socket for the ball of the head of the capitate in this movement. The capitate with the hamate, lesser multangulars and metacarpals rotates 50° around a transverse axis through the middle of the head of the capitate: as the curve of the navicular capitellum is on a circumference relative to this centre, the multangulars move on to the most volar portion of this facet. It is there-



Text-fig. 1. Composite tracing from röntgenograms of flexion and extension. The bones of the forearm were fixed. The exposures for flexion and extension were made on separate films and later compounded. The result is self-explanatory.

fore apparent that the greater part of the flexion movement takes place at the intercarpal joint, and that disease of this joint leads to a greater disability than does a lesion of the radiocarpal joint.

Extension is also a compounded movement (Plate I, fig. 6, and text-fig. 1). At the radiocarpal joint the lunate and triquetrum move through 28° and come to face posteriorly and distally. The navicular swings with and on the lunate until its long axis is in line with that of the forearm; it never moves posteriorly to this line. The anterior disposition of the radiocarpal ligament and the association with the tendon of the flexor carpi radialis muscle are responsible for this limitation. The distal carpal row moves 16° on the axis transversely through the centre of the head of the capitate. The volar surface of the socket for the head of the capitate is formed by the strong ligament joining the triquetrum and hamate, and by the distal facet of the dorsally tilted lunate bone. The multangulars slide on to and even beyond the most proximal portion

of the capitellar facet. When this movement is completed, the most distal point of the carpus is the capitellum of the navicular with the hook of the hamate slightly proximal to this plane. Forced extension accentuates this position very slightly. In falling on to the extended hand the force is taken directly on to the small head of the navicular.

SUMMARY

Radial deviation occurs entirely at the transverse carpal joint.

Ulnar deviation is almost wholly a sliding movement of the radiocarpal joint.

Flexion and extension are compounded of radiocarpal and transverse carpal joint movements with the latter the more extensive.

The navicular bone shows a wide range of movement in relation to the other bones of the proximal carpal row.

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EXPLANATION OF PLATE I

Fig. 1. A lateral röntgenogram of the wrist in the straight (not flat) position. The position of the lunate bone (*L*) with the navicular (*N*) inclined at an angle to it are shown. The capitate and hamate are slightly dorsally inclined. The greater multangular is shown on the middle of the capitellar facet of the navicular.

Fig. 2. Antero-posterior röntgenogram of wrist in straight position. Note relation of the lunate to the radius and that of the long axis of the capitate to the radius. The navicular is foreshortened due to volar flexion; it slightly overlaps the capitate due to rotation on its longitudinal axis.

Fig. 3. Antero-posterior röntgenogram in ulnar deviation. The change of relation between the lunate and radius is evident: the navicular has come into line with the radius. The rotation of the capitate around the axis through the centre of the head is shown. The multangulars have moved in an ulnar direction on the capitellum of the navicular. Approximation of the hamate to the lunate produces splaying of the proximal row of carpals.

Fig. 4. Antero-posterior röntgenogram of the wrist in radial deviation. The lunate bone has much the same relation to the radius as in the straight position but the capitate has moved much relative to the lunate carrying the hamate with it. The navicular is shown foreshortened due to further volar flexion and the multangulars have moved radially on the navicular.

Fig. 5. Lateral view of wrist joint in full flexion. The edge of the navicular has been drawn in black; full flexion of this bone and the capitate with little change in the position of the lunate (*L*) is shown. The greater multangular has moved on to the most volar portion of the navicular capitellum.

Fig. 6. Lateral röntgenogram of wrist in full extension. The movement of the lunate is seen with the navicular (drawn in white line) approximately in line with the bones of the forearm. The capitate is fully extended and the greater multangular has moved proximally and dorsally on the navicular capitellum.

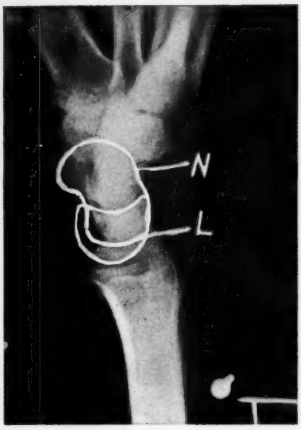

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

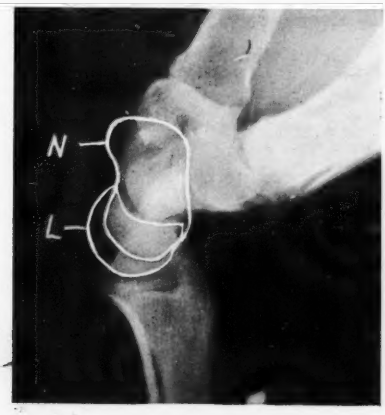

Fig. 6.

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THE CHANGES IN THE ALVEOLAR AND DEMILUNE CELLS OF THE SIMPLE AND THE STIMULATED PARALYTIC SUBMAXILLARY GLAND OF THE CAT

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INTRODUCTION

In a previous report (Rawlinson, 1933) evidence was presented of relationships between the sympathetic and parasympathetic nerves, and the secretory activity of the demilune and alveolar cells respectively. In a search for further evidence the paralytic gland was studied and also the effect upon it of sympathetic and adrenalin stimulation.

The spontaneous salivary secretion from the submaxillary gland after section of the chorda tympani was named by Claude Bernard the "paralytic secretion". Heidenhain (1868), Langley (1885) and Gerhardt (1903) made short studies of the histological appearance of such glands, and Maximow in 1901 reported a comprehensive investigation of the paralytic gland in dogs. He found that the alveolar cells became decreased in size, and though some areas showed intensive and disruptive changes, most of the cells were apparently in a resting stage (except in one early case of 9 days' duration). Changes in demilunes were more difficult to estimate. He considered them to be always active in the paralytic gland, being first enlarged, later becoming decreased in size and possessing less "fuchsinophile granules".

METHODS

The submaxillary gland of the cat was used. The lingual nerve with its accompanying chorda tympani was sectioned close to the mandible. The main fixative used was formol-Zenker, while haematoxylin and eosin, and combined iron haematoxylin-mucicarmine-aurantia stains were applied. The unoperated gland was used as an immediate control, even though it undoubtedly suffers some changes.

OBSERVATIONS

The following observations deal with (*a*) the uncomplicated condition of the paralytic gland, (*b*) the paralytic condition with superimposed sympathetic and adrenalin stimulation. The record here is of observations on the demilune and alveolar cells; the striated ducts being discussed elsewhere (Rawlinson, 1935).

(*a*) *The paralytic gland*

The gland when removed was always strikingly decreased in size, confirming previous observations. The sections show that the shrinkage takes place in the parenchyma of the gland, since the connective tissue is somewhat increased in amount.

Alveolar cells. In the first 3 days the alveolar cells show signs of moderate activity. These are the presence of irregularities in the cytoplasmic network, fairly general but small vacuoles, and nuclei more spherical than normal. After the end of the first week the findings are similar to those reported by Maximow. The cells get much smaller in size, but otherwise appear to be entirely normal and non-active. There are also the areas which he mentions in which the changes are more intensive (Plate I, figs. 1, 2). By the sixth week the alveolar cells are very small, less than one-half of their normal size, but beyond this their appearance is normal. As with the striated ducts there is a slight tendency in the control gland to develop the same changes, but it is not marked.

Demilune cells. Our results support Maximow's conclusion that these cells are active, but in our material from the cat we are able to assay these changes more accurately.

In the first week the activity is quite general. It is shown in the general increase in the distinctness of the cytoplasmic network, and in the development in it of irregularities and small vacuoles. These changes are at first quite general in the operated gland, and occur to a definite degree in the control. From the second week on, the changes in these cells in the paralytic gland present a wider range, and the signs of activity, though to some extent general, are as Maximow records, most marked in scattered areas. In such places the cells develop an empty appearance not exactly similar to vacuolation, because the clear areas are not sharply limited but grade off into the scant basal cytoplasm. For the next two or three weeks these changes continue, and they show more uniformly if not as intensively in the control. After the fourth week the control gland presents a fairly normal appearance, but in some animals changes can still be seen in it at later stages. The paralytic gland at this time shows areas of intensive change but the demilunes undergo a fairly general decrease in size and differentiation between them and the alveolar cells becomes more difficult due to the decrease in the eosinophilic material. With iron haematoxylin it can be seen that the mitochondrial content is much less than in the normal or in the control gland cell (cf. Maximow's "decrease in fuchsinophile granules"). In addition the nucleus is smaller and stains darkly.

From the fourth week on to the end of our series (43 days), the changes in the demilunes of the operated gland progress toward a state in which the majority of the demilunes are very small, often appearing simply as a rim around the alveolar cells, a decrease in size much more drastic than that suffered by the alveolar cells. The nuclei are small and dark and often extremely shrunken, the mitochondria are very scarce, and those that occur are in the

form of very fine granules. The small cytoplasmic area is clear, and has mere traces of the material of the cytoplasmic network. However, during the later stages, there can still be found in varying numbers larger demilunes that are still in the earlier stage and have a loose network. The control gland shows demilunes that can be easily differentiated from the alveolar cells. They average less than normal size, but are still considerably larger than the average cell of the paralytic. The mitochondria are much more numerous and coarser, and the majority of the nuclei are fairly large and stain exceedingly lightly in sharp contrast to the small dark nuclei of the operated gland.

Lumen content. The alveolar lumen is slightly dilated in the early stages and the intercellular capillaries show up more frequently, but it is difficult to make out any special content in them. After the second week the alveolar lumina and the intercellular channels present for the most part a normal appearance.

The cells lining the striated ducts undergo marked changes in the paralytic gland that can be interpreted as secretion, exhaustion and recovery phases. These are described elsewhere, but the content of the ducts is of interest here. In the first week, and to a lesser degree in the second, the ducts always contain an exceptional amount of yellow staining material sometimes mixed with a quantity of black staining substance. These elements are evidently contributed by the cells of the ducts themselves. Added to this is a considerable amount of material forming clear spaces interspersed in the stainable substances. Material staining with mucicarmine is quite scarce, and when seen stains very weakly as if diluted. When the striated duct cells show the washed-out appearance of the exhaustion phase the lumina are fairly clear of stainable material. This lack of visible content continues throughout the phase of exhaustion, in which the lumina become quite wide due to the great decrease in the height of the lining cells. As the recovery takes place, the ducts develop their normal narrow lumen and occasional small amounts of mucoid content. Thus a dilute non-staining element is the chief content of the ducts in the paralytic gland.

Interstitial tissue and blood vessels. Most of the paralytic glands of the third or fourth week show a remarkable increase in the interstitial tissue, but after this time the hyperplasia is not so marked. The blood vessels are choked with cells in the early stages. Later this feature is not so obvious, but in the last experiment the vessels after 43 days do not yet present a normal appearance.

Summary. The paralytic gland shows at first a generalised activity which includes alveolar cells, demilune cells, and also, as reported elsewhere, the cells of the striated ducts. After a few days the alveolar cells atrophy, while the demilunes continue to be active until they become very small and they do not, in the time covered by our series (43 days), undergo any recovery. It is interesting to note that in such sustained activity the clear areas of the cell become extensive, but are not sharply delimited into vacuoles as in the previously reported stimulation experiments. This points to a loss of

basic cytoplasmic material. The excretory channels contain mostly a clear non-staining element.

(*b*) *Stimulation of the paralytic gland*

Such a type of experiment as this seemed to offer possibilities. Maevsky (1923) found that the threshold of stimulation for the sympathetic was lowered in the operated gland, and Fleming and MacIntosh (1934) showed that the stimulation of the sympathetic in a paralytic gland would produce a greater amount of secretion than normally. In view of this increased excitability, we performed five experiments in which paralytic glands were subjected to cervical sympathetic or intravenous adrenalin stimulation. In most cases a piece of tissue was removed from the gland before the experiment and used as a control. The glands used came from two 9-day, and from 16-, 18- and 43-day experiments. The histological findings follow.

Striated ducts. These ducts present the typical changes found in paralytic glands of such stages (Rawlinson, 1935), and these are sufficiently intense to make it difficult to see any superimposed change. There is, however, one notable feature in the presence, sometimes very marked, of a peculiar mucoid staining material in the cells. This same condition has been previously found in certain chorda stimulation experiments. It was only found in two of the five of the present series, but in them it was marked.

Alveolar cells. In the two 9-day experiments these cells show signs of moderate activity similar in kind to those noted in other experiments (irregularity, vacuole formations, dilatation of lumen, etc.). The glands from the other experiments show the alveolar cells quite normal for paralytic glands of those durations, with the exception of occasional isolated cells.

Demilune cells. These are most certainly intensively active in all experiments. They vary in size from medium to quite small, and the nuclei also vary but the majority are crumpled up. The mitochondria become quite scarce, and the granular material in the cell is tremendously decreased, so that after weak stimulation the cells have an empty look while, with increased stimulation, the cells are markedly vacuolated (Plate I, figs. 3, 4, 5). The changes are marked in every case even with a degree of stimulation that would not give definite changes in a normal gland. It is remarkable that in glands of later stages, where the demilunes become very small, they will quickly develop a vacuolated appearance under stimulation, as though such a development were due to the inability of an exhausted cell to cope with the demands made upon it.

The gland passages contain a little dilute mucoid material in the two early paralytic glands, but in all the experiments the main content is a clear element whose presence can be inferred from the moderately dilated lumina, and the way in which the moderate amount of stainable material is honeycombed with clear areas.

Summary. The results show definitely that the demilunes of the paralytic gland have been markedly affected by sympathetic and adrenalin stimulation.

The striated ducts are active, but it cannot be ascertained whether their activity is increased over that due to the nerve section alone. The alveolar cells are excited to a varying extent in the early paralytic gland, but are practically unaffected in later stages.

DISCUSSION

The results of these experiments support our previous suggestion that the demilune cells are predominantly under the control of the sympathetic mechanism, and the alveolar cells under the influence chiefly of the parasympathetic fibres. The supporting evidence comes from the signs that the alveolar cells of the paralytic gland, after a short preliminary stage of activity no doubt due to the irritative effects of nerve section, enter on a stage that we, in common with Maximow, take to be a resting one. The decrease in size may be due to the loss of a trophic (tonus) mechanism. On the other hand, the demilune cells continue to be active. This steady activity evidently reduces their basic cytoplasmic material to such an extent that they are easily exhausted by any superimposed stimulation, for when sympathetic or adrenalin stimulation was added to the effect of nerve section, the demilune cells showed marked vacuolation in every one of our experiments. As pointed out previously (1933) this phenomenon occurring in gland cells during such experiments as these seems to represent an exaggeration of normal secretory processes. There are good grounds for believing that marked vacuolation, when appearing under such conditions, is a sign of excessive stimulation of the secretory processes in the cells in which it occurs. On such grounds the results of sympathetic and adrenalin stimulation on the paralytic gland point to the more or less special effect of such stimulation on the demilune cells.

The content of the gland channels both in the uncomplicated and the stimulated paralytic gland is mainly a clear material. A careful consideration of the histological material from these and other experiments leads to the opinion that the output of the demilunes is of a thin consistency, and contributes materially to the non-staining content of the duct lumina so prominent in the sections from these experiments. Langley (1885) observed, and we have also noticed, that the saliva secreted by the submaxillary gland of the cat in response to sympathetic stimulation is more watery than chorda tympani saliva. This adds further support to the hypothesis of a sympathetic-demilune mechanism.

These observations throw no light on the problem of the hyperexcitability of the demilune cells in the paralytic gland. This problem and that of the production of the changes in the unoperated gland, probably lie in the field of local neurochemical substances.

CONCLUSIONS

Histological observations on the cat's submaxillary gland during the paralytic secretion, as well as after the excitation of such paralytic glands by sympathetic and adrenalin stimulation, add further support to the idea that

the secretory effect of the sympathetic is mainly on the demilune cells, while that of the parasympathetic is similarly exercised chiefly on the alveolar cells.

In carrying out the physiological part of this investigation the author has relied on the advice and help of Prof. B. P. Babkin, which he has generously supplied.

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EXPLANATION OF PLATE I

The figures are all photomicrographs of sections fixed in formol-Zenker and stained with iron haematoxylin, mucicarmine and aurantia.

Fig. 1. Area (×350) from a 9-day paralytic gland, showing the edge of an area of intense activity. In the upper part of the figure the cells are irregular, vacuoles are frequent and a group of alveolar cells in the centre of the field contain only a small amount of finely granular cytoplasmic material. In the lower part of the figure the cells are approximately normal, although the demilune cannot be sharply differentiated from the alveolar cells.

Fig. 2. Area (×550) from centre of fig. 1. The sparse granular cytoplasm and abundant clear areas of the alveolar cells can be seen.

Fig. 3. Area (×450) from a 9-day paralytic gland stimulated by intravenous injections of adrenalin. There is some irregularity of the alveolar cells and some dilatation of the alveolar lumens. The main feature is the abundance of the large clear vacuoles in the demilune cells.

Fig. 4. Area (×450) from an 18-day paralytic gland after sympathetic and adrenalin stimulation. The demilunes are vacuolated while the alveolar cells are practically unaffected.

Fig. 5. Area (×450) from a 45-day paralytic gland stimulated via the sympathetic for 1½ hours. Note the vacuolation of the demilunes and the unaffected alveolar cells.

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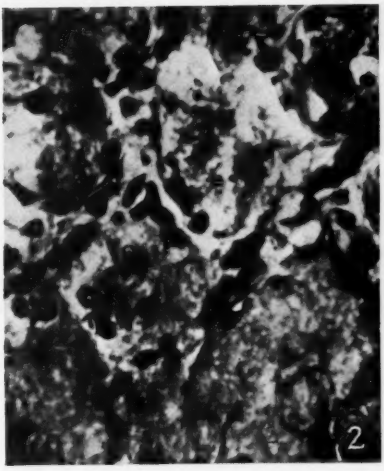
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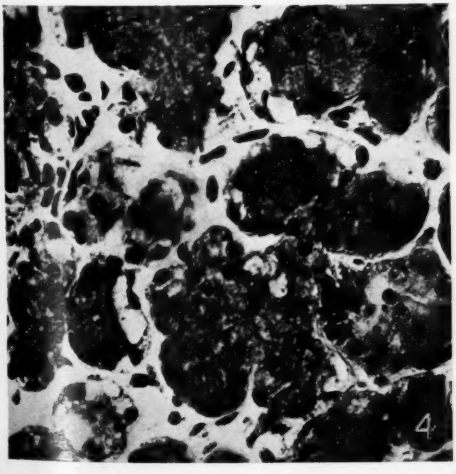
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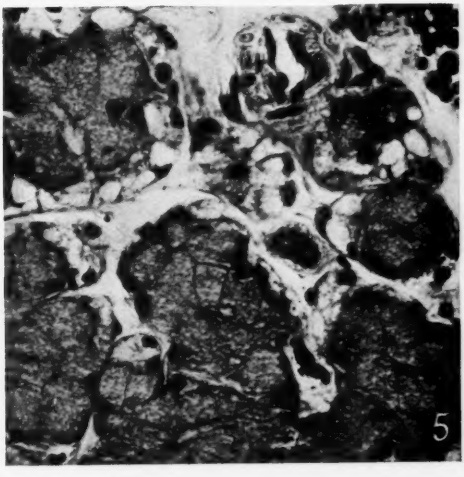
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PATTERNS OF THE AORTIC ARCH IN A SERIES OF 133 MACAQUES

By CHARLES F. De GARIS

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The present report is based on a study of the aortic arch in 133 macaques, including the following species[1]: *Pithecus rhesus* 115, *P. sinicus* 10, *P. fascicularis* 3, *P. irus* 2, *P. mordax* 2, *P. nemestrinus* 1. From this distribution of species it is seen that while the material as a whole suffices for comparative study of variations within the genus *Pithecus*, only one species, *rhesus*, is represented by a series large enough for significant statistical treatment. Accordingly it is proposed to note as to classification and incidence the aortic patterns in the various species of *Pithecus* and to examine statistically the distribution of these patterns in the species *rhesus*.

The animals from which these aortic specimens were taken have been received from various sources. A great many specimens of *P. rhesus* were from the Carnegie colony supervised by Dr C. G. Hartman; many of this and other species were sent by Prof. J. F. Fulton, New Haven; several of *P. rhesus* were made available through the efforts of Drs S. S. Tower and H. A. Howe. The hearts and aortic arches of all these specimens were kindly placed at my disposal by Dr A. H. Schultz, who receives and records contributions to the Hopkins Anatomical Collection. To the above contributors and others my thanks are due.

PATTERNS AND INCIDENCE

The patterns of aortic branches in macaque are, in so far as practicable, described and figured here in the order of their frequency in Man, thus retaining pattern designations similar to those used in a recent report on the human aorta (De Garis, Black and Riemenschneider, 1933). The series, as here arranged in Fig. 1, arbitrarily starts with the normal human sequence of three separate trunks and, passing through various stages of migration of the a. carotis communis sinistra upon the anonyma, reaches the prevailing mammalian pattern of a long truncus communis, in all of which stages the a. subclavia sinistra remains as a separate aortic stem.

[1] The terminology employed here is from D. G. Elliot (*A Review of the Primates*, 1913). The priority and adequacy of much of this terminology, not only as to the various species of macaques, but also as to the generic name *Pithecus*, is sharply questioned by Gerrit S. Miller, Jr. (*The Anatomy of the Rhesus Monkey*, 1933). According to him the term *Pithecus* should be replaced by *Macaca*, and it is his view that the following terms for species are synonymous: *Pithecus rhesus* = *Macaca mulatta*; *P. sinicus* = *M. radiata*; *P. fascicularis* and *P. irus* (also *Macacus cynomolgus*) = *M. irus*; the terms *mordax* and *nemestrinus* are retained.

Series similar to the above have been figured by Keith (1895) and Parsons (1902). In the Keith series are included twenty-two specimens of macaque (*nemestrinus, cynomolgus* and *arctoides*), in that of Parsons nine (species not noted). In most respects the Keith sequence corresponds to the one figured here, a striking exception being his placement of the subclavia sinistra at an increasing distance from the anonyma as the carotis communis sinistra approaches the human type of three separate trunks. There would, indeed, seem to be some *a priori* connection between widening of the interval between subclavia sinistra and anonyma as the carotis communis sinistra migrates down the anonyma to occupy this interval. Yet the singular fact is that the subclavia sinistra bears no expected or explicit relation to the other aortic stems, such as the Keith sequence indicates. In many cases of the long truncus communis (C_2) the subclavia sinistra is widely separated from the anonyma, while in most cases of the human type (A) the subclavia sinistra is closely crowded against the carotis communis sinistra.

The Parsons' series, in so far as it includes the macaques, figures the subclavia sinistra widely spaced from the other aortic stems, and there is nothing in his text to indicate that he attaches any importance to this spacing as a basis for classification of patterns. Considering the above-mentioned fact that the subclavia sinistra is so diversely spaced, it is apparent that a classification based on this spacing would conflict with, and preclude, the more obvious and significant categories based on origin of the carotis communis sinistra. Accordingly no effort is made here to represent the subclavia sinistra in any but its most frequent spacing.

For statistical treatment the materials of Keith and Parsons cannot be accurately separated, because Parsons has included some of Keith's specimens and has added some of his own. For practical purposes any patterns of the Parsons series not figured by Keith are regarded as new for that series, and are so treated. The excellent descriptions of the arterial circulation in "*Macacus cynomolgus*" and "*Macacus sinicus*" by Rojecki (1889) are unsuitable for statistical treatment because variations of the aortic stems are not cited in reference to species or to numbers of specimens. His only figure (13) of the aortic arch depicts a long truncus communis comparable to C_2 of the present fig. 1.

Below are given brief descriptions of the various aortic patterns as found in my own material (see fig. 1); also explicit notations are made of the number of specimens of each species in which a given pattern is found. Insistence on the fact that all the material described here is new, i.e. belongs to an original series not in any part borrowed from the work of other authors, will be appreciated by those who have attempted to deal in a statistical way with the literature of the subject.

Pattern A. This pattern exemplifies the normal human sequence of aortic stems, viz. from right to left: (1) anonyma, (2) carotis communis sinistra, (3) subclavia sinistra.

This arrangement is found in fourteen specimens of *P. rhesus*.

Pattern B. This pattern, one of the most frequent variants of the human aorta, is characterised by a radix communis for anonyma and carotis communis sinistra, i.e. this latter artery arises in, and thus obliterates, the left angle made by intersection of the anonyma and the convexity of the aortic arch. The only practical test of this arrangement as a distinctive pattern is to open the aortic arch along its concavity and turn it inside out, thus demonstrating whether or not the anonyma and carotis communis sinistra have a common exit from the aorta.

Pattern B is found in fifteen specimens of *P. rhesus*.

Pattern C. This pattern anticipates the prevailing mammalian arrangement of aortic stems, and is a frequent variant in Man. It consists of but two trunks from the aortic arch, the first a truncus communis for anonyma and carotis

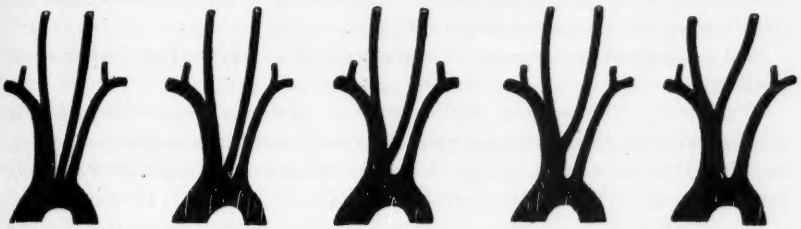

Fig. 1. Diagrams of aortic patterns in macaque: A, the usual human arrangement of three separate stems in right-to-left sequence: (1) a. anonyma, (2) a. carotis communis sinistra, (3) a. subclavia sinistra; B, an arrangement very frequent in Man, comprising a radix communis for anonyma and carotis communis sinistra; C, another arrangement frequent in Man, comprising a short truncus communis for anonyma and carotis communis sinistra; C_1, a truncus communis intermediate in length between that in Man and that prevailing in Mammals; C_2, a long truncus communis such as prevails in Mammals.

communis sinistra, the second the subclavia sinistra. In the present series of macaques there is much diversity in length of the truncus communis, only the short truncus being comparable to the pattern of the common trunk in Man.

Since in this connection it is desirable to define the word "short" in some numerical terms, comparable as between Man and macaque, a basis of comparison was established as follows: twenty human aortic arches were measured as to inside diameter of cross-sections taken at the level of attachment of the ligamentum arteriosum; their average diameter was found to be 24·5 mm. Likewise twenty aortic arches from *P. rhesus* were measured at the same point as to inside diameter; their average was 4·8 mm. There was thus established for the aortic diameters of Man and macaque a ratio of approximately 5 : 1. Then in thirty-four human aortic arches having the truncus communis (pattern C) the longest truncus was found to be 28 mm., as measured from the convexity of the aortic arch to the vertex of the angle between carotis communis sinistra and anonyma. Reducing this length of 28 mm. by the ratio of

5 : 1, it is found that a truncus communis about 6 mm. long would for macaque be the upper limit of pattern C as compared to Man. While this is but a rough approximation, it is at least better than a comparison based on the mere impression of similarities in configuration. Accordingly this pattern of the short truncus communis (maximum length 6 mm.), is designated C without subscript, since it corresponds to the same pattern in Man.

Pattern C is found in sixty-two specimens of *P. rhesus* and in one specimen of *P. nemestrinus*.

Pattern C_1. This pattern is characterised by a truncus communis of intermediate length, i.e. one more than 6 mm. and not over 11 mm. long. It is found in twelve *P. rhesus*, two *P. fascicularis* and one *P. sinicus*.

Pattern C_2. This pattern, the prevailing mammalian arrangement, has a long truncus communis, i.e. one more than 11 mm. in length, the longest in the series being 17 mm. This pattern is found in nine *P. rhesus*, five *P. sinicus*, two *P. irus*, two *P. mordax* and one *P. fascicularis*.

All subsequent patterns are enumerated in the polygons of frequency as "others".

Pattern C_3. This pattern, with a truncus communis of variable length, is peculiar in that the truncus divides trichotomously into subclavia dextra, carotis dextra and carotis sinistra, thus eliminating the anonyma. This arrangement is found in two specimens of *P. sinicus*.

Pattern C_4. This pattern, an extension of the preceding, exemplifies the arrangement found in many of the Felidae and in certain of the Ungulata and Marsupialia in that the truncus communis gives off the subclavia dextra as its first branch and continues as a further common stem for the aa. carotides communes. This pattern is found in two specimens of *P. sinicus* (in one of which the bicarotid stem is less than 2 mm., in the other 3+mm.) and in one specimen of *P. rhesus* (bicarotid 4+mm.). In the specimen of *P. rhesus* there is a long slender branch arising from the dorsal aspect of the aortic arch just beyond the subclavia sinistra and passing upward with the n. recurrens sinistrus to the larynx and the glandula thyreoidea. By reason of this contribution to the thyreoid the foregoing arrangement may well be considered in relation to the next group of patterns.

Patterns F_2 *and* F_3. The F patterns in the human series, as described by De Garis *et al.* (1933), were those characterised by an a. thyreoidea ima coming either directly from the arch of the aorta between anonyma and carotis communis sinistra (F) or from the anonyma (F_1). There is no exact counterpart of either of these human patterns in the present series of macaques. However, in one specimen of *P. rhesus* having a long truncus communis there is a slender thyreo-thymic artery arising in the angle between the anonyma and carotis communis sinistra and passing upward and to the left to supply the thymus and the glandula thyreoidea (pattern F_2). In another specimen of *P. rhesus* having a radix communis (pattern B), there is a sizable artery arising from the carotis communis sinistra 11 mm. cephalad to the aortic arch and apparently

distributed to areas which in man are supplied by the a. thyreoidea inferior. It should be recalled that in members of the genus *Pithecus* and in many other primates the a. thyreoidea inferior is usually lacking. Patterns of the thyreoidea ima are frequent in the chimpanzee, and have been reported by Keith (1895) in two macaques.

SUMMARY OF INCIDENCE

A summary of incidence of the above patterns in the genus *Pithecus* is shown in the polygons of frequency (fig. 2). The solid black polygons represent the absolute numbers of specimens of *P. rhesus* alone; the polygons with cross-hatching represent additional numbers in the present series comprising species other than *rhesus*; the empty polygons surmounting these represent the numbers of macaques added from the literature (Keith and Parsons). To facilitate the direct comparison of species other than *rhesus* and the group taken from the literature, two other sets of polygons are constructed on separate base-lines. Thus in fig. 3 (1) is shown the incidence of aortic patterns in species other than *rhesus*; in fig. 3 (2) is shown the incidence in macaques added from Keith and Parsons.

It is seen from fig. 2 that for the genus *Pithecus* as a whole the pattern of the short truncus communis (C) must be considered the norm from which variations are to be computed. On the basis of a total of 162 macaques from all sources it is found that seventy or 43·21 per cent. fall within this norm. It is further seen that variants to the left of this norm are those with distinctly human patterns (A and B), and comprise a total of thirty-six (22·22 per cent.), while variants to the right of the norm are those with prevailing mammalian patterns (C_1 and C_2) or with aberrant patterns of various kinds ("others"), the combined total of those to the right of the norm being fifty-six (34·56 per cent.). Whence the total of variants, i.e. on both sides of the norm, is found to be ninety-two (56·79 per cent.). It would thus appear from fig. 2 that for the genus *Pithecus* as a whole the arrangement of aortic branches occupies a position intermediate between the human patterns on one side and the prevailing mammalian pattern on the other side.

However, when additions from the literature and species of the present series other than *rhesus* are considered separately as to incidence of aortic patterns, i.e. arranged in polygons of frequency on separate base-lines, as in fig. 3 (1) and (2), then wholly incongruent polygons are obtained. The species other than *rhesus* (fig. 3 (1)) are seen to have the pattern of the long truncus communis (C_2) as their norm, with a relatively strong modal value and with but one specimen in the human range for the truncus communis (C). Since there are only eighteen specimens in this group, statistical treatment here cannot be regarded as significant. Still of this number it may be recalled that ten are *P. sinicus*, which very consistently shows one or another of the following closely related patterns: the long truncus communis with carotis sinistra far removed from the aortic arch and near the carotis dextra (C_2); the

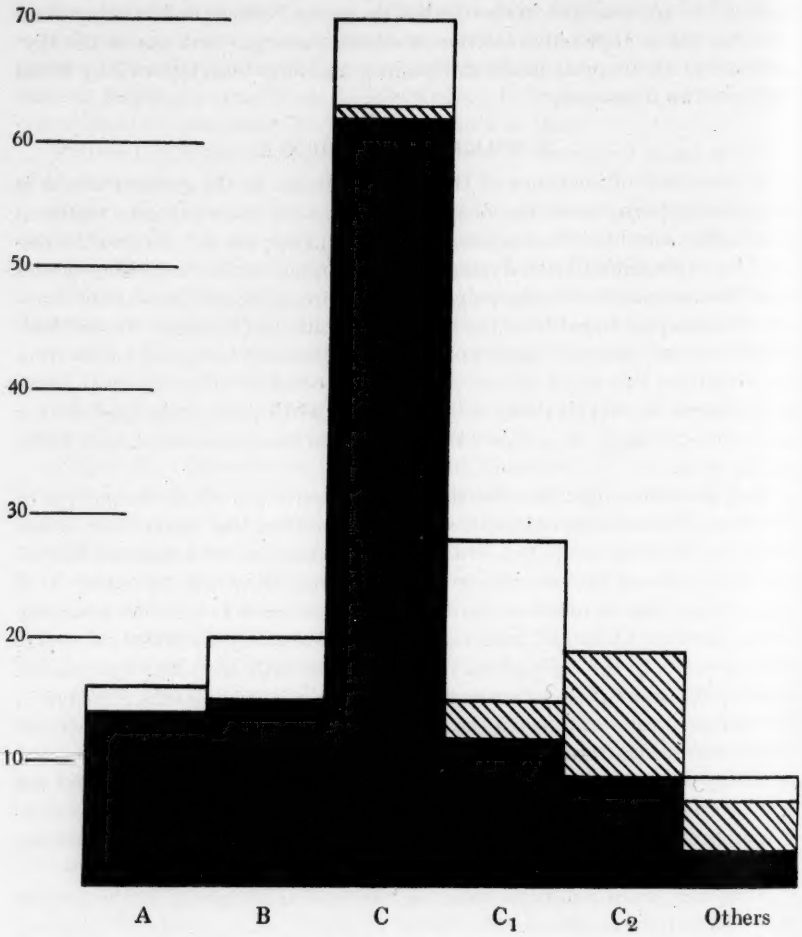

Fig. 2. Frequency polygons of aortic patterns in macaque, showing the absolute numbers of specimens exhibiting each of the patterns A to C_2. Specimens with patterns C_3, C_4, F_2 and F_3 are included in the polygons designated "others". The solid black polygons represent pattern frequencies in *P. rhesus* alone. Polygons with cross-hatching represent additional numbers in the present series comprising species other than *rhesus*. Empty polygons surmounting these represent additions from the literature (Keith and Parsons). The summations of polygons thus afford a composite picture of pattern frequency in 162 macaques, twenty-nine of which are from the literature, 133 being the present series, of which latter 115 are *P. rhesus*.

truncus communis of variable length dividing trichotomously into subclavia dextra and the two carotides (C_3); the truncus communis giving off the subclavia dextra as its first branch and continuing as a bicarotid stem (C_4). Thus are exemplified in *P. sinicus* the various stages of apparent migration cephalad of the carotis sinistra to, and beyond, the level of the subclavia dextra. These same stages are also clearly demonstrated in a series at hand of the aortic arch in domestic cat (*Felis domesticus*, forty-two specimens). The similarity of aortic patterns in *P. sinicus* and *F. domesticus* is, indeed, very striking, while

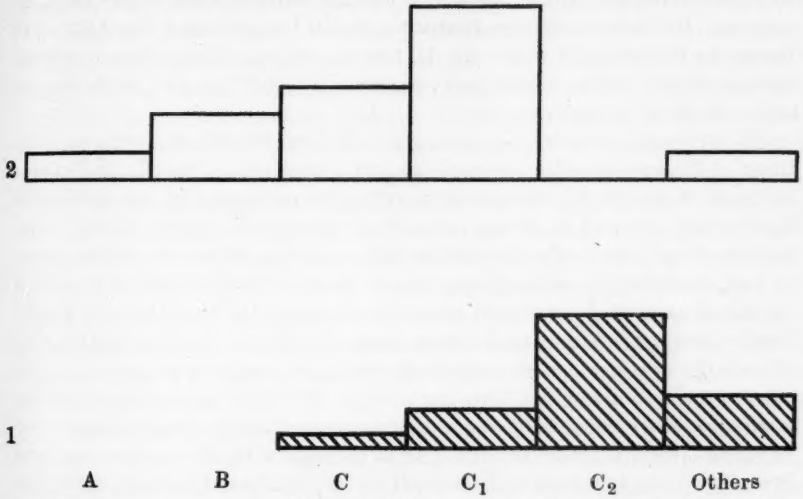

Fig. 3. (1) Frequency polygons with cross-hatching represent the absolute numbers of aortic patterns in macaques of the present series other than *P. rhesus*. There is none in this series with pattern A or pattern B. The one specimen with pattern C is *P. nemestrinus*; the three with C_1 are one *P. sinicus* and two *P. fascicularis*; the ten with C_2 are five *P. sinicus*, two *P. mordax*, two *P. irus* and one *P. fascicularis*; the four with "other" patterns are *P. sinicus*. Total of species other than *rhesus*, 18. (2) Frequency polygons without cross-hatching represent aortic patterns in various species of macaque as figured by Keith and Parsons. Total 29. The foregoing polygons, as here constructed on separate base lines, facilitate direct comparison of the groups in question, while these same polygons, added to those of *P. rhesus* (fig. 2), express the combined incidence of aortic patterns in the genus *Pithecus* as a whole.

the dissimilarity of aortic patterns in *P. sinicus* and the other species of *Pithecus* serves to place *sinicus* in a unique position in the present series. In this series the number of specimens within the norm is ten (55·55 per cent.), the number outside the norm eight (44·44 per cent.).

The incidence of aortic patterns in the twenty-nine specimens from Keith and Parsons (fig. 3 (2)), yields a set of frequency polygons radically different from those of the present series other than *rhesus*, not only in the position of the norm, but also in the direction of the variants. Among these twenty-nine specimens there is a norm of strong modal value about pattern C_1, the truncus

communis of intermediate length. It should be said in this connection that since both Keith and Parsons employ very much reduced diagrams, and since these are the sole bases on which one may judge the classification of their material, it is largely a matter of personal impression whether the truncus communis, as figured by them, is intermediate or long. Yet even if other commentators choose to place the norm at C_2 rather than at C_1, the modal value would remain the same and the variants in any case would be preponderantly on the human side of the norm. Of these twenty-nine specimens thirteen or 44·82 per cent. are within the norm, leaving sixteen or 55·17 per cent. as variants. It is interesting to note that the Keith-Parsons series (fig. 3 (2)), and the genus *Pithecus* as a whole (fig. 1), though varying about different norms, show strikingly similar per centum variations, i.e. 55·17 per cent. in the former series, 56·79 in the latter.

When frequency polygons of the genus *Pithecus* (fig. 2) are compared with those of the species other than *rhesus* and with those of the Keith-Parsons series (fig. 3 (1) and (2)), it is readily seen that the contour of fig. 2 is dominated by the large series of *P. rhesus*, not only as to the norm and its modal value, but also largely as to the distribution of variants on either side of the norm. It may, therefore, be seriously questioned whether the frequency polygons of the genus as a whole are based on a fair sampling. The fact that the Keith-Parsons series, containing no *P. rhesus*, shows practically the same distribution of variants as the composite series representing the genus would seem to indicate that the sampling has been fair enough. Yet if the preponderant species were some other than *rhesus*, for instance *sinicus*, there is strong reason to believe that the norm would be shifted far to the right of the human patterns, and it would be simply a matter of chance if the distribution of variants fell within the corresponding range of some other mixed series of *Pithecus*.

In this connection the point to be urged is briefly this, that the statistical treatment of variation within a genus, regardless of numbers or method of sampling, is a composite treatment, where dissimilar units (species) are brought together and made to assume relations, as expressed by averages, percentages and the like, which have little or no significance for the genus itself. The three sets of polygons offered here bear out this point explicitly. They are not congruent—it would be an accident if they were—because the things they represent have no necessary relation. It may well be a matter of interest to compare range, or degree, of variation in series of dissimilar units (species), where mathematical congruence is not a desideration. In fact, that is all the foregoing comparisons have thus far achieved.

But when one turns to a single species, i.e. a series of like units, such as the 115 specimens of *P. rhesus*, then mathematical congruence does become a desideration. Given another series of the same species, the same norm should hold and with a similar modal value; also the distribution of variants should be similar. Further the similarity should become closer and closer as the number of samples in each series is increased, until with very large numbers the factors

of similarity on which membership in the species depends should be expressed in a very close approximation to mathematical congruence. In the present series of 115 *P. rhesus* (solid black polygons of fig. 2) the norm at pattern C has a strong modal value, there being sixty-two specimens or 53·91 per cent. at the norm, and fifty-three specimens or 46·08 per cent. as variants on the two sides of the norm, of which latter twenty-nine or 25·21 per cent. are to the left or human side, twenty-four or 20·86 per cent. to the right or mammalian side.

With a series as large as this, comprising samples of a single species, it is possible to draw certain fairly definite conclusions. Thus it may be said that for *P. rhesus* the norm of aortic branches is a truncus communis within the range of 6 mm. maximum length; that this norm is within the range of comparable length for the human truncus communis; that this norm stands intermediate between the distinctly human patterns on one side and the distinctly mammalian pattern on the other; that variations are almost equally distributed about the norm. Given another large series of the same species one would expect all the foregoing conclusions to hold, not of course in exact detail, i.e. not as to the actual modal value of the norm, and therefore not as to an exact numerical expression for the variants. But in general the frequency polygons of any series as large as this, or larger, should show a high degree of congruence with these polygons, unless there should happen to be something special about the samples. In view of the diverse sources of the present material there is nothing to suggest that these samples are in any way selected or otherwise special.

It would be a matter of interest to compare the extent of variation in *P. rhesus* with that of other series of mammals, if the materials were available. But for some reason there is a dearth of literature on these subjects. The careful dissections of 106 aortic arches and subclavian arteries in rabbit by Baldwin (1920) are not presented in a way to render the material accessible for statistical treatment. My own series of aortic arches in cat and dog are as yet not large enough for significant treatment. There is already abundant material reported on the aortic arch in Man, but since a further large series of my own is still to be studied, it seems best to defer comparison until this series can be included.

SUMMARY AND CONCLUSIONS

Patterns of branching of the aortic arch, studied in a series of 133 macaques (115 *P. rhesus*, ten *P. sinicus*, three *P. fascicularis*, two *P. irus*, two *P. mordax*, one *P. nemestrinus*), show the normal human sequence of aortic stems in fourteen *rhesus*; the radix communis for anonyma and carotis sinistra in fifteen *rhesus*; the short truncus communis (maximum 6 mm.) for anonyma and carotis sinistra in sixty-two *rhesus* and one *nemestrinus*; the intermediate truncus communis (maximum 11 mm.) in twelve *rhesus*, one *sinicus*, two *fascicularis*; the long truncus communis (maximum 17 mm.) in nine *rhesus*, one *fascicularis*, two *mordax*, two *irus* and five *sinicus*. Other variants include

the a. thyreoidea ima in two *rhesus*, trifurcation of the truncus communis into subclavia dextra, carotis dextra and carotis sinistra in two *sinicus*, and the truncus communis giving rise to a bicarotid stem in one *rhesus* and two *sinicus*. A series of twenty-nine macaques from Keith and Parsons is added to the above in constructing frequency polygons for the genus *Pithecus*. This method of statistical treatment is examined critically. Polygons for *P. rhesus* alone lead to the following conclusions: the norm is the short truncus communis, this being within a range comparable to the human truncus communis; this norm stands intermediate between distinctly human and distinctly mammalian patterns; variants are almost equally distributed on either side of the norm. In the composite series *P. sinicus* alone shows marked divergence in the direction of patterns prevailing in the Felidae.

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FURTHER ABNORMALITIES IN THE BLOOD VASCULAR SYSTEM OF FROGS

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The following is an account of various abnormalities in the blood vascular systems of specimens of *Rana temporaria* that have been encountered during class dissections. I have to thank my colleagues for directing my attention to some of them. As will be seen, they all involve major arteries or veins, and four of them, all venous abnormalities, were found by examining the dissections left after a class examination when about two hundred frogs were used.

The arterial abnormalities are interesting, since they occur far less frequently than those in the veins. As in previous records (8, 9) the term "adult" is used to indicate that the frogs were sexually mature but not of full size, and "fully grown" implies that the animals had about reached the limit of growth.

1. *An abnormal occipital artery arising from the systemic arch.* This is a full-grown male specimen of *R. temporaria*, which in all other respects appeared to be normal.

An abnormal artery arises from the right systemic or aortic arch at about the level of the "carotid gland" on the carotid arch but before the ligamentum caroticum joins the aortic arch. It runs antero-laterad, crosses the ventral surface of the internal jugular vein and passes dorsad to become the occipital artery. It soon divides into the orbito-nasal and temporal arteries which seem to have normal relationships.

In the normal frog the occipital artery is a branch of the occipito-vertebral artery which arises from the aortic arch just anterior to the point of origin of the subclavian. The vertebral artery (A. vertebralis dorsi) arises in the normal position of the occipito-vertebral artery, and gives off a ramus cranialis. The present abnormal artery takes the place of the occipital artery. The normal occipito-vertebral artery arises from the embryonic, paired dorsal aorta, a remnant of part of which persists as the ligamentum caroticum. The artery here described, however, arises ventral to the ligamentum caroticum. In *Salamandra salamandra* there is an arteria pharyngea ascendens which (see Francis (3)) arises from almost the same position on the systemic arch. It is not distributed in quite the same manner, however, and is related to ventral not dorsal vessels.

No similar abnormality appears to have been recorded in the Anura.

2. *An abnormal subclavian artery.* This specimen is an adult male *R. temporaria* apparently normal in other respects.

The coeliaco-mesenteric artery (A. intestinalis communis) arises from the union of the two aortic arches in the normal manner, and the left subclavian artery originates from the aorta just postero-lateral to it. The subclavian passes forwards parallel with the left aortic arch until it reaches its normal level, and then turns laterad to the arm having a typical distribution. Attention was directed to this abnormality in the first place because, until fully dissected, it appeared as if the left aortic arch was double.

It is difficult to give a reason for this abnormality, for no similar vessel appears during the course of development, and the cause may perhaps have to be sought on phylogenetic, not ontogenetic grounds. Two facts are perhaps of some significance in this connection. In the Elasmobranchs there seems little doubt that the subclavian artery is derived from an ordinary parietal or segmental artery that is situated conveniently to supply the pectoral fin, as was suggested by Dohrn (2), and it is not always the same segmental artery on the two sides (see O'Donoghue and Abbott ((10), p. 855)). So that phylogenetically the subclavian artery is to be regarded as a segmental. In the *Urodela* according to Bethge (1), Francis (3) *et al.*, the subclavian artery originates from the single dorsal aorta posterior to the union of the aortic arch.

No similar abnormality appears to have been recorded from the *Anura.*

3. *The absence of a right anterior vena cava.* This is a full-grown male *R. temporaria*, and so far as can be seen normal in other respects.

On the left side the external jugular, innominate and subclavian veins are formed in the usual manner, and by their junction constitute the left anterior vena cava. Also upon the right side these three veins unite but, instead of forming a normal anterior vena cava, they end blindly at their junction and no such vessel is present. The blood is conveyed from the right to the left sides of the head by means of two transverse anastomoses. One of these passes ventrad across the bases of the truncus arteriosus posterior to the level of the glottis and joins the points of union of the external jugular, innominate and subclavian arteries on each side. It is thin-walled and narrower at the two ends than in the middle. The second crosses anterior to the glottis from the external jugular just below its formation on the left to the base of the lingual vein on the right. On the right side the lingual and mandibular veins open separately into the junction of the left external jugular and the anterior anastomosis.

No example of such a double anastomosis has been recorded previously so far as is known.

4. *The absence of an anterior vena cava and an innominate vein.* This is a full-grown male *R. temporaria*, and has certain other abnormalities of the anterior veins in addition to the absence of a right anterior vena cava, but in other respects it appears to be normal.

On the left side the innominate and subclavian veins are constituted and join in the normal manner. The left external jugular vein is dilated at its posterior end just before it joins the union of the innominate and subclavian to form a typical anterior vena cava. The mandibular vein enters its anterior end, and so does a transverse anastomosis from the right external jugular. The left lingual vein enters this anastomosis just before the latter joins the external jugular.

On the right side the external jugular vein is formed by the confluence of the lingual and mandibular veins, and a short way down it receives the anastomosis. After this it swells out to form a dilatation similar to that on the left side, and is then joined by the subclavian vein. No anterior vena cava is formed on this side, and the blood from it is returned to the heart via the anastomosis and the left external jugular vein. There is no innominate vein

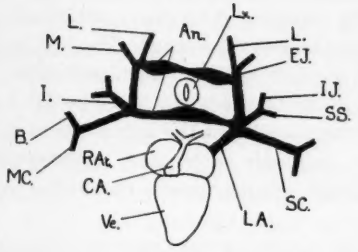

Fig. 1.

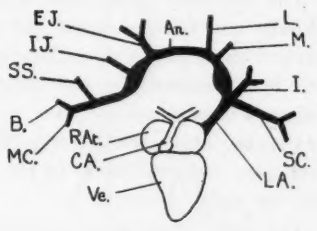

Fig. 2.

Fig. 1. Diagram of the heart and anterior veins viewed from the ventral side. The lengths of the anterior vena cava and the corresponding space are exaggerated for diagrammatic purposes. *R. temporaria* ♂, Example 3.

Fig. 2. Diagram of the heart and anterior veins viewed from the ventral side. The lengths of the anterior vena cava and the corresponding space are exaggerated for diagrammatic purposes. *R. temporaria* ♂, Example 4.

on the right side, and the internal jugular enters the external jugular in the anterior part of the dilatation, while the subscapular vein enters the subclavian about half-way along its course. It is to be borne in mind that, in a number of abnormal frogs with a persistent posterior cardinal vein, the internal jugular and subscapular veins enter separately into the modified innominate.

5. *The absence of an anterior vena cava and a posterior vena cava.* This specimen is an adult female *R. temporaria* which has no anterior vena cava on the right side, no posterior caval vein, a persistent right posterior cardinal vein and unusual conditions in the renal portal veins.

On the left side the external jugular, innominate and subclavian veins are normally constituted, with the exception that a transverse anastomosis enters the external jugular and they join to form a normal anterior vena cava. On the right side the same three veins unite but, while the first and last are formed

in the typical manner, the innominate vein receives the persistent right posterior cardinal vein and the internal jugular and subscapular veins open separately into it. No anterior vena cava is present on this side, and consequently the blood is returned by an anastomosis passing from right to left external jugular veins. This anastomosing trunk is slightly more posterior than it is generally in this type of abnormality, for instead of lying anterior to the glottis it is actually ventral to it.

The inter-renal vein is fairly normal but, instead of continuing forward as a posterior caval vein, it bends round the anterior margin of the right kidney and joins with the continuation of the right renal portal vein to form the persistent posterior cardinal vein. This vessel passes forwards on the dorsal wall of the body cavity, lateral to the aortic arch, dorsal to the subclavian vein and then turns postero-mesiad to become the innominate vein receiving the subscapular and internal jugular veins separately as noted above.

The left renal portal vein is normally constituted by the junction of the ischiadic and femoral veins, but is much thinner than usual. The left pelvic vein is slightly enlarged, so that the bulk of the blood from the hind-limb must have been returned by the abdominal vein. The reduced renal portal vein receives a dorso-lumbar factor, but its blood supply to the kidney must have been small. This is reflected by the fact that only three small venae renales revehentes carry its blood to the inter-renal vein as against four larger ones from the right kidney.

The right renal portal vein is formed in the typical manner, but passes along the dorso-lateral margin of the kidney with only a slight diminution in size until, at the anterior end, it joins with the inter-renal to form the persistent posterior cardinal vein. It receives a large dorso-lumbar branch. In spite of the fact that the renal portal vein is continued along the kidney, the presence of four venae renales revehentes on this side indicates that there was a portal supply and the right kidney is slightly larger than the left.

It follows from the peculiar arrangement of the blood vessels that have just been described that all the blood from both hind-limbs, save that carried by the abdominal vein, and all that from the kidneys and dorso-lumbar regions had to go to the right side of the head, and then be returned by the left anterior vena cava to the heart, a truly extraordinary state of affairs. This specimen is unique in two respects. First, it exhibits a combination of an absence of a right anterior vena cava and also of a posterior caval vein: this has not been recorded previously. Secondly, it is a female. There have now been recorded a number of instances of the presence of anastomoses between the internal jugular veins in *R. temporaria*, and in the eleven examples where this is definitely known to be associated with the absence of an anterior vena cava and the sex is known it is always the right side that is involved and the frog is always a male. Two of the examples described above are precisely similar, but the third, while lacking a right anterior vena cava, is a female. This shows that such an abnormality can occur in the female, but there does not appear to be

any explanation of the disproportion between the sexes in which it has been recorded, i.e. thirteen males to one female, nor why the abnormality should always be on the right side. The example recalls to some extent that recorded by Holle (4), but this differed in several important respects. It was a female, but *R. esculenta*; the anterior vena cava was absent on the left side; the posterior caval vein was present, but the abdominal vein was double at the anterior end, each part opening into the corresponding subclavian vein.

No explanation of the disappearance of the right anterior vena cava and the appearance of the anastomosis is yet forthcoming. It is obvious however that, if for any reason the anterior vena cava fails to develop on one side, some such arrangement for the return of blood from that side must be developed. There are normally anastomoses between the tiny veins in the region between the two external jugulars as elsewhere, and possibly one of these is dilated to make provision for the greatly increased flow. In a fairly primitive Elasmobranch, *Scyllium canicula* (see O'Donoghue (7)), there is normally an anastomosis between the inferior jugular sinuses which lies in approximately a corresponding position.

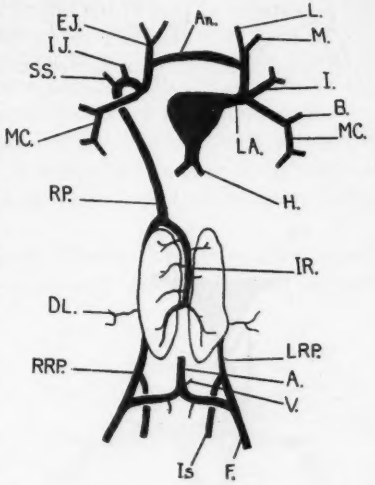

Fig. 3. Diagram of the principal veins viewed from the ventral side with the heart removed to expose the sinus venosus. *R. temporaria* ♀, Example 5.

The reason for the persistence of the posterior cardinal vein will be dealt with below when other examples are considered.

6. *An abnormal abdominal vein.* This specimen is an adult female *R. temporaria*, which in other respects appears to be normal.

The term "anterior abdominal vein" is commonly employed in English text-books but it is open to the objection that "anterior" is used in the sense that it has in human anatomy, i.e. to mean ventral. If a qualifying adjective is used "median" would be preferable, but no such qualification appears necessary, since only one abdominal vein is present and, moreover, without qualification one would presume that it was mid-ventral in position. A qualifying word is necessary in animals like the Elasmobranchs where two abdominal veins are present, and this is met by terming them lateral abdominals.

The abdominal vein is normally constituted at the hinder end, and it pursues a typical course up to the level of the Xiphisternum where, instead of passing deep to enter the hepatic portal vein, it goes on forwards to the right

of the gall bladder. It penetrates the pericardial cavity, along the wall of which it runs for a short distance and then turns abruptly postero-mesiad to enter the junction of the posterior vena cava and the sinus venosus.

No such abnormality appears to have been recorded previously, and no explanation of its occurrence can be given. The continuation of the abdominal vein anterior to the hinder end of the Xiphisternum might be looked upon as the persistence of part of the lateral abdominal on that side (see O'Donoghue ((8), p. 192)), but this fails to account for its penetration of the pericardial cavity and its entering the posterior caval vein. It is probable that the retention

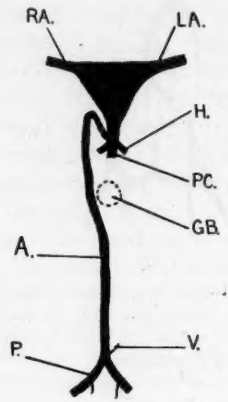

Fig. 4.

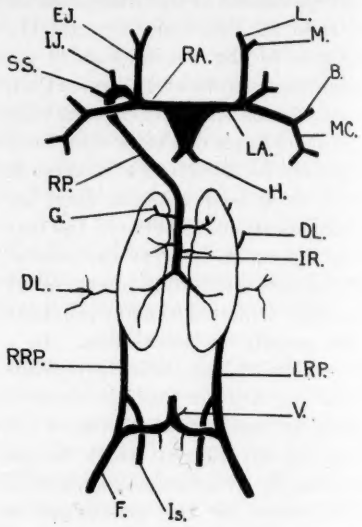

Fig. 5.

Fig. 4. Diagram of some of the veins viewed from the ventral side with the heart removed to expose the sinus venosus. *R. temporaria* ♀, Example 6.

Fig. 5. Diagram of the principal veins viewed from the ventral side with the heart removed to expose the sinus venosus. *R. temporaria* ♀, Example 7.

of the anterior ends of one or both of the lateral abdominal veins present in the tadpole is more common than is suggested by the number of examples recorded, since an abnormal vessel in this position would be very likely to be destroyed in opening the frog for ordinary class dissection.

7. *A persistent right posterior cardinal and absence of a posterior vena cava.* This is a full-grown specimen of a female *R. temporaria*, which is apparently normal in other respects save for the presence of unusual factors to the left renal portal vein.

The anterior veins on the left side are normal in constitution and unite to form a typical anterior vena cava. The external jugular and subclavian veins

on the right are normal, but the innominate is the continuation of the persistent posterior cardinal vein. It is noticeably dilated and the internal jugular and subscapular veins open separately into it. The renal portal veins are typical on both sides, and end on the dorso-lateral margins of their respective kidneys so that they are both fully portal. On the right the renal portal vein receives a dorso-lumbar factor in the usual position, but on the left there are in addition two more anterior factors coming from the dorsal body wall which open into the front portion of the renal portal vein. The inter-renal vein is normally constituted, but at the anterior end, instead of continuing as the posterior caval vein, it bears away to the right, runs parallel with the aortic arch and then sweeps round laterally and mesially as the dilated innominate vein.

This specimen is somewhat similar to, but not identical with previously recorded examples of this abnormality. It brings the number of instances of a posterior cardinal vein persisting on the right side in *R. temporaria* to fifteen.

8. *A persistent left posterior cardinal vein.* This specimen is an adult, nearly full-grown male *R. temporaria*, which is apparently normal in other respects save that the right renal portal vein is double at the anterior end.

All the anterior veins are normal, save that the left innominate is the continuation of the persistent posterior cardinal vein. The inter-renal vein continued as the posterior caval vein is typical, except that what appears to be the anterior vena renalis revehens continues on across the front end of the left kidney into the persistent cardinal. Both renal portal veins are normally constituted, as is the abdominal vein. The right renal portal vein bifurcates a short distance behind the kidney and the two branches enter that organ separately. The left renal portal vein continues on along the latero-dorsal border of the kidney, diminishing in size, but leaves the anterior corner of that body, where it receives a factor from the inter-renal vein, to become the persistent cardinal. This vessel runs forwards parallel with the aortic arch, bends laterally and then posteriorly to become the innominate into which the internal jugular and subscapular veins open separately. Although the left renal portal vein gets noticeably smaller as it passes along the kidney and there are the

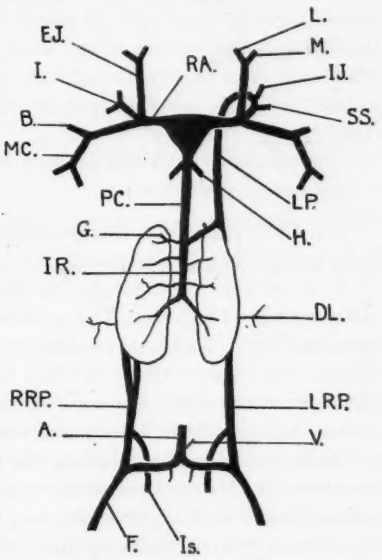

Fig. 6. Diagram of the principal veins viewed from the ventral side with the heart removed to expose the sinus venosus. *R. temporaria* ♂, Example 8.

normal number of venae renales revehentes indicating a portal supply, the left kidney is distinctly smaller than the right.

This specimen is somewhat like that recorded by O'Donoghue, Example 24 ((8), p. 201), save that the factor from the inter-renal is smaller and the right renal portal vein is split.

9. *A persistent left posterior cardinal vein and absence of a posterior caval vein.* This specimen is an adult male *R. temporaria*, apparently normal in other respects.

The anterior veins are normal, save that the left innominate is the end of the persistent posterior cardinal into which the internal jugular and subscapular veins open separately. The inter-renal vein is fairly typical, save that it does not continue forward as the posterior caval vein but ends abruptly. Its blood is conveyed by a large trunk in the position of the anterior vena renalis revehens across the ventral face of the front end of the left kidney into the cardinal vein. This passes forwards, bends first laterally and then mesially to become the innominate which is dilated at its end after the point of entrance of the internal jugular vein. The right renal portal and abdominal veins are normal; the left renal portal is constituted in the ordinary way but passes along the latero-dorsal margin of the kidney to become the persistent cardinal. It retains the same calibre throughout, and there is only one vena renalis revehens, so that the left kidney is largely or entirely non-portal and is smaller than the right.

This specimen is so similar, save in sex, to O'Donoghue, Example 25, that it does not need illustration.

10. This specimen is a large full-grown male *R. temporaria*, which appears to be normal in other respects.

It is on the whole similar to the foregoing specimen, so that only the differences need be noted. The inter-renal vein is short, not continued into the posterior caval, and receives three venae renales revehentes from the right kidney; the most anterior of these has an ordinary genital factor. About a third of the way from the anterior end the inter-renal vein passes across the ventral surface of the kidney and opens into the "renal portal vein". Close to the lateral edge of the kidney the transverse vessel receives a vena renalis revehens, which runs forwards from near the hinder end of the kidney. All the adrenal tissue in this part appears to be confined to the narrow strip between this revehent vein and the margin of the kidney. The left kidney is thus non-portal and is much smaller than the right. The left renal portal vein is normally constituted, but does not actually touch the kidney; it passes dorso-laterad of that organ and comes towards it at the point where the factor from the inter-renal vein enters. Thereafter it is attached to the kidney margin by connective tissue, but is readily dissected away. It receives a normal dorso-lumbar factor, and a short distance anterior to the kidney a well-marked genital vein.

This arrangement of inter-renal and renal portal veins has not been described previously.

11. This is an adult male *R. temporaria*, which appears to be normal in other respects.

It is similar to no. 9 above in most respects, but the left renal portal vein diminishes slightly in size as it passes along the kidney and the venae renales revehentes on both sides are more or less typical. The inter-renal vein does not cross the ventral surface of the kidney but curves around its anterior end. The left kidney is probably partially portal and a little, if any, smaller than the right.

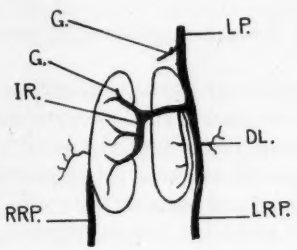

Fig. 7.

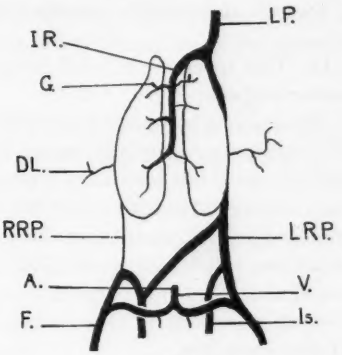

Fig. 8.

Fig. 7. Diagram of the veins related to the kidney viewed from the ventral side. *R. temporaria* ♂, Example 10.

Fig. 8. Diagram of the principal posterior veins viewed from the ventral side. *R. temporaria* ♂, Example 13.

12. This is an adult male *R. temporaria* and, as far as observed, normal in other respects.

It is also similar in most ways to no. 9 above, save that the inter-renal vein starts on the right kidney, and does not receive its first revehent vein from the left kidney until about half-way along, and this vein has a marked factor from the posterior end of the kidney. The inter-renal curves round the anterior end of the kidney. The left renal portal vein runs along the margin of the kidney, but this organ is apparently non-portal and smaller than the left.

13. This specimen is a full-grown male *R. temporaria*, and in addition to the points noted has also an abnormality in the renal portal system but is otherwise normal.

Once again the general condition resembles that of no. 9 above, but the inter-renal vein passes around the anterior end of the kidney as in nos. 11 and 12. The renal portal vein on the left side is normally constituted, but before

joining the kidney it receives an abnormal factor from the right side. The left femoral and pelvic veins are normal. The left ischiadic vein, however, just before it joins the femoral, gives off a large vessel which passes obliquely antero-laterad across the body cavity dorsad of the two common iliac arteries and the 8th and 9th spinal nerves. It turns ventrad between the 8th and 7th spinal nerves and enters the left renal portal vein just before this reaches the kidney. The left renal portal vein is formed by the confluence of the femoral and ischiadic veins, but is a minute vessel; it runs to the kidney, on the margin of which it receives a small ordinary dorso-lumbar and an additional small factor. Both kidneys appear to be only partly portal and are approximately equal in size.

No such abnormality appears to have been recorded.

14. This specimen is a full-grown male *R. temporaria*, apparently normal in other respects.

The anterior and posterior veins except the left innominate are normal, so that both kidneys are fully portal, and they are equal in size. The inter-renal vein is typical, but instead of continuing forward as the posterior caval vein it bends away to the left parallel with the aortic arch, as the persistent posterior cardinal vein. At the anterior end it turns laterad and posterad to become the innominate vein which is not dilated, and into which the internal jugular and subscapular veins open separately. On the left side the dorso-lumbar vein arises more anteriorly than usual, but opens into the renal portal at the ordinary level.

In general this specimen recalls those recorded by O'Donoghue ((5), p. 368, (6), p. 138, (8), p. 205) save that the posterior cardinal vein persists on the left side and not on the right as in these examples, and also it is not a female like the last of them.

The preceding eight examples, i.e. nos. 7–14, have all persistent posterior cardinal veins. The main drainage of the posterior body region in the tadpole is carried out by the posterior cardinal veins which originate in the bifurcation of the caudal vein, and their development and subsequent history in *R. temporaria* have been worked out by Shore (12) and their bearing on the present type of abnormality suggested previously (O'Donoghue (8), p. 211). It is only necessary to state here that, instead of disappearing as they do normally, one or other of them has persisted. In seven of them this has been necessitated apparently by the posterior caval vein failing to develop, but this is not so in no. 8, and it is difficult to suggest why the cardinal vein has remained in this specimen.

Wholly or partially persistent posterior cardinal veins have been recorded from various *Anura*. In *R. temporaria* there have been described previously thirty-four specimens and the eight here bring the total up to forty-two. In four of these part or the whole of the cardinal vein persists on both sides; in fourteen on the right and in twenty-four on the left. From the manner in

which the posterior caval vein develops it might be expected that the cardinal vein would persist more frequently on the left. This is borne out by the numbers given, confirmed by the other *Anura* and by no. 8 above, where it is the left posterior cardinal vein that is present, although a posterior caval vein is formed. What is more striking, however, is the unequal distribution between the sexes for, in the forty-two frogs, the sex of four is not on record and of the remainder thirty-one are males and seven females. This cannot be explained from the developmental history of the vessels themselves. The pronephros is intimately associated with the posterior cardinal vein, indeed its tubules actually project into the vein. The oviduct is developed from folds in the dorsal coelomic wall which start immediately by the pronephros and pass backwards quite close to the position occupied by the posterior cardinal vein. It may be that the development of the oviduct in this region tends to inhibit the retention of a posterior cardinal vein.

15. *Abnormalities of the renal portal system.* This specimen is an adult male *R. temporaria*, and is apparently normal in other respects except the liver.

Both renal portal veins and the abdominal vein are normal in origin and that of the left normal in relation to the kidney. The right renal portal vein passes along the latero-dorsal margin of the kidney, hardly diminishing in size, and curves round the anterior end of that body to open into the posterior caval vein. The inter-renal and posterior caval veins are normal.

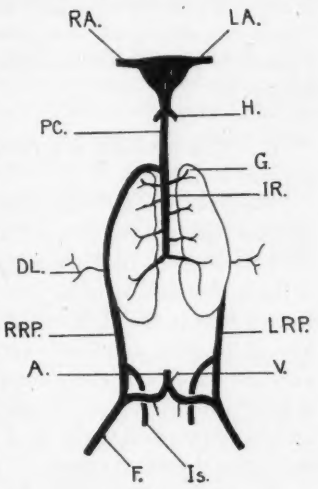

Fig. 9. Diagram of the principal veins viewed from the ventral side with the heart removed to expose the sinus venosus. *R. temporaria* ♂, Example 15.

The right lobe of the liver is reduced to a small mass roughly triangular in shape, with two of its edges 5 mm. long and the other 2·5 mm. The left lobe of the liver is enormously enlarged and extends across on to the right side of the body cavity. Normally the left lobe is approximately triangular in shape with one angle anterior and about a third of the way back starts a deep cleft, the incisura intralobaris, which passes obliquely postero-laterad dividing it into anterior and posterior moities. Thus the anterior portion is lateral and the posterior part ventro-mesial in position. In the present example the incisura intralobaris commences in the usual position, but curves mesially to the right extremity of the lobe so that the anterior portion of the lobe is first lateral and then passes ventrad to the posterior part. The posterior part also runs across to the right side of the body and is largely divided by a deep fissure, not

represented in the normal liver, which commences right at the front apex of the lobe and runs approximately parallel with the anterior edge of the posterior portion but does not reach its right-hand end. Thus a large part of the posterior portion is actually in front of the anterior part across the ventral region of the body cavity. The normally constituted abdominal vein lies ventrad of the mesial face of the anterior portion of the left lobe of the kidney, and turns dorsad through the incisura intralobaris to join the hepatic portal vein.

As far as the abnormal renal portal vein is concerned this specimen recalls that recorded by Shore (11), but it is on the right instead of the left. No such malformation of the liver appears to have been recorded.

16. This specimen was observed by the late Prof. Dunkerley, who kindly sent me a sketch and notes thereon. It was an adult *R. temporaria*, but the sex was not recorded.

No abnormality of the liver was noted, but otherwise the specimen agrees closely with that just described. The difference is that at the point where the right renal portal vein curves round the antero-lateral corner of the kidney it receives a factor coming from the dorsal body wall some way anterior to it. This is probably to be interpreted as the retention of that part of the posterior cardinal vein between an anterior parietal factor and the front end of the kidney.

17. This specimen is a full-grown male *R. temporaria*, apparently normal in other respects.

The renal portal veins and the abdominal vein are normally constituted and the right related to the kidney in the usual manner. The left renal portal vein, however, instead of going to the kidney, passes obliquely forward ventrad of the ureter and the kidney, between the testes and ventrad of the duodenum. It then runs to the right of the gall bladder along the mesial border of the left lobe of the liver, and finally turns dorso-mesiad to enter the bridge of the liver near the left lobe slightly anterior to the hepatic portal vein. The left kidney is thus non-portal and is smaller than the right. The blood from the left hind-limb is all returned to the liver either by the right pelvic and abdominal veins or by the abnormal vessel.

No similar abnormality has been recorded, and it does not seem possible to give an explanation of how such a vessel could be formed.

18. This specimen is a full-grown female *R. temporaria* and, so far as observed, normal in other respects save for the calcareous glands.

The posterior veins on the right and the inter-renal are normal. On the left side the renal portal vein is constituted normally and runs to the postero-lateral margin of the kidney, but here, instead of pursuing a normal course, runs obliquely antero-mesiad across the ventral surface of that body to enter the inter-renal vein in the place of the posterior vena renalis revehens, whose

tributaries it receives. The dorso-lumbar vein on the right is as usual. On the left side there are two dorso-lumbar veins; one is fairly normal but small, and the other is anterior to it and larger. This unusual dorso-lumbar vein is derived from the confluence of a number of conspicuous factors on the dorsal body wall which are not present in the normal frog. When it reaches the kidney it turns sharply posterior and runs along the ventral face of the kidney, receiving the ordinary dorso-lumbar vein, and continues till it enters the renal portal vein just after this has reached the kidney. This longitudinal vessel, which conveys the blood from the dorso-lumbar veins to the renal portal, appears to give no factors to the kidney. This organ seems to have no portal supply and is distinctly smaller than that on the right particularly at its posterior end. The vessel on the kidney might be regarded as a part of the normal renal portal vein, but usually this is on the dorsal surface. All the calcareous glands of this specimen are large, and those in the region of the left dorso-lumbar veins are enormous.

No such condition has been recorded previously.

19. This specimen is an adult female *R. temporaria*, and appears to be otherwise normal.

The abnormality concerns the left renal portal supply. The ischiadic vein, when it reaches the point where it usually joins the femoral, sends off a small anastomosis to that vein, but continues forwards as an independent trunk to reach the postero-lateral border of the kidney at the place where the renal portal vein usually reaches it. The anastomosis is of much smaller calibre than the main trunk. The ischiadic vein continues on the latero-dorsal border of the kidney like a normal renal portal. The femoral vein gives off a normal pelvic vein, and then proceeds to the kidney, but does not enter the renal portal until about half-way along.

This arrangement does not appear to have been recorded.

20. This specimen is an adult male *R. temporaria*, and the only abnormality appears to be that of the left renal portal supply.

The femoral vein is usually dorso-lateral in the leg, but in this example it is lateral and, after giving off the pelvic vein, runs forward to the kidney in a normal manner. At first sight it looked as if the ischiadic vein was absent, but further dissection showed this not to be so. The ischiadic is actually double in the thigh, one branch lying on each side of the ischiadic nerve. At the upper end of the thigh the two branches join and enter the femoral vein just before this gives off the pelvic; thus this junction is posterior to its ordinary position.

While doubling or looping of various veins has been described before (see O'Donoghue (8), p. 217), it has not been noted in the ischiadic vein.

21. This specimen is an adult male *R. temporaria*, and the only abnormality observed concerns the constitution of the right renal portal system.

On the right side the femoral vein is completely absent in the section

between the entrance of the vena circumflexa femoralis medialis prima and the point of origin of the pelvic vein, so that at first sight the femoral vein appears to be absent. Dissection shows, however, that it turns dorso-mesiad at the normal point of entrance of the vena circumflexa femoralis medialis prima and passes dorsal to the femur, the ischiadic artery, ischiadic nerve and the ramus posterior of the latter to enter the ischiadic vein. At the point where it turns, it receives a tributary from in front which apparently drains the region normally drained by the factors entering the femoral vein in the portion here missing. The deleted portion makes it appear as if the pelvic vein arises from the ischiadic, but actually there is a small turn at the beginning of the pelvic which suggests that the first part of it is actually the persistent femoral vein between the usual junction of the femoral and ischiadic veins and the point of origin of the pelvic vein.

No similar abnormality has been found described in the literature.

Normally, when the mesonephroi develop they grow into the hinder sections of the posterior cardinal veins, thus forcing them into loops, one around each kidney with irregular spaces, sinusoids, joining them. The further growth of the mesonephroi break down the antero-lateral and postero-mesial portions of the loop, thus forcing the blood to pass through the sinusoids from the lateral vessel, sometimes termed Jacobson's vein, to the mesial vessel. This establishes a portal supply to the kidney, and Jacobson's vein is the renal portal. The two mesial limbs of the loops unite in the middle line and so form the inter-renal portion of the adult posterior caval vein. Some of the abnormalities in the renal portal systems recorded above and elsewhere can be understood by assuming that these developmental changes did not occur in the normal manner. Thus, in the foregoing examples, nos. 5, 15 and 16 illustrate the failure of the growing right kidney to break through the antero-lateral part of the loop. Nos. 18, 13 and perhaps 8 indicate a failure of the left kidney to break the postero-mesial part of the loop. No. 13 suggests that the postero-mesial portions of the two loops joined, thus establishing a channel from right to left sides, which persisted even when the immediately anterior parts of each portion disappeared. Nos. 10 and perhaps 17 indicate a more deep-seated disturbance of the loop formation.

Examples like nos. 19, 20 and 21, however, cannot be explained in this manner, for the abnormalities do not occur in the actual derivatives of the posterior cardinal veins themselves. They result from derangements in the formation of the limb vessels or the manner in which they enter the posterior cardinal veins, concerning which we have little or no information. It is not possible at present to offer any reasonable explanation.

22. This specimen is a male *R. temporaria.* Although it is larger than many typical male frogs that have reached maturity, it is still not fully mature and has not passed through a breeding season. There is no sign of a nuptial pad with its roughened pigmented surface, even though the first digit is slightly

larger than that of a female of the same size. The testes are quite small, the corpora adiposa represented by thin strands of fatty tissue and the vesiculae seminales are also small. All these organs are smaller than in the normal male of the same size during the resting period after spawning. From its general appearance it suggests that it is a year late in reaching full sexual maturity, although this does not appear to be in any way related to the abnormal heart. The other blood vessels, save in so far as they are directly affected by the unusual condition of the heart, are normal. I have to thank Prof. Weymouth Reed of St Andrews University for kindly sending me this specimen, which was one of a number being used for class work.

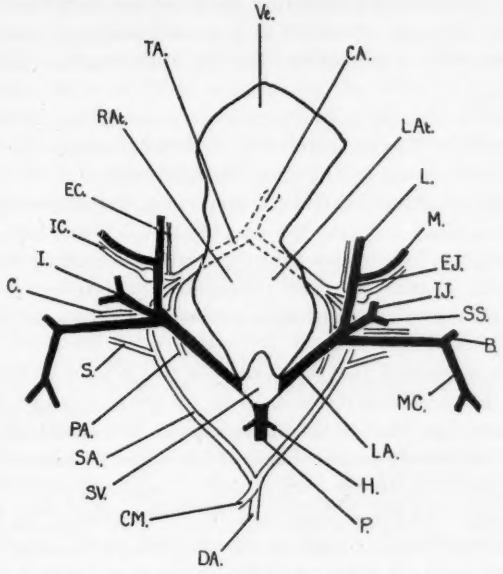

Fig. 10. Diagram of the heart and the principal veins and arteries viewed from the ventral side. *R. temporaria* ♂, Example 22.

The heart is an elongated structure, 17 mm. long, lying practically in the middle line, although the bulk of the anterior end is slightly to the left. It is divided near the middle by a shallow constriction. The anterior end is the thick-walled ventricle which is not of the normal triangular shape but roughly rectangular, and it reaches forward to within a short distance of the symphysis menti. The posterior moiety is formed by the atria and the sinus venosus. The latter is a roughly triangular sac which projects ventrally from the caudal end of the heart but passes, with practically no line of demarcation, into the right atrium. Right and left atria are divided by a septum atriorum which is practically vertical and median in position, so that the two atria lie right and left and not left-dorsal and right-ventral as in a previously recorded specimen

(O'Donoghue(8), p. 182). The two pulmonary veins open together into the hinder end of the left atrium and the sinus venosus receives the right and left anterior and the posterior caval veins.

The conus arteriosus leaves the ventricle at its posterior end slightly to the left of the middle line. It is not quite as long as in the normal heart and, of course, passes backwards instead of forwards. The truncus arteriosus pass obliquely latero-posterad, but the three arterial arches, carotid, systemic and pulmocutaneous and their proximal branches occupy practically normal positions. The external jugular, innominate and sub-clavian veins come from their normal places, but unite a little posterior to the usual level to form the anterior venae cavae, which, since they have to pass right back to the hinder end of the heart, are not only out of their normal positions but are noticeably longer. They run postero-mesiad to enter the sinus venosus with the posterior caval vein.

As far as can be seen there is no membrane passing from the dorsal surfaces of atria or ventricle to correspond with a dorsal mesocardium such as was found in a previous example (O'Donoghue(8), p. 183).

The internal structure of the heart, apart from the abnormal relationships of its cavities, appears normal. My son, Charles O'Donoghue, who saw this specimen when alive, informs me that the heart was beating normally except that the systolic contraction was propagated postero-anteriorly. This, of course, is to be expected from the reversed positions of the atria and ventricle.

It has been suggested that the cause of the flexion of the heart is the inelasticity of the pericardium which forces the growing heart tube to bend in order to accommodate itself to the limited space. The condition of the present example and a previously recorded one (O'Donoghue(8)) throws doubt on this explanation, since in both the pericardium is about double the normal size and shows no sign of having prohibited the elongation of the heart. Indeed it would appear as if the pericardium adapts itself to the growth of the heart rather than *vice versa*. A more probable explanation appears to be that the anterior and posterior ends of the heart tube are more or less held in position by the vessels leaving and entering them. These vessels in turn are more or less fixed by the relative positions of the structures they serve. If the differential growth transforming the primitive heart tube into the adult heart is accompanied by an increase in length, as it most markedly is, and there is no corresponding increase in the distance between the points where arteries and veins join the adjacent tissues, then it follows that the heart must become flexed. The present example shows that this can be overcome to a certain, but not a sufficient extent, by an elongation and displacement of the anterior venae cavae.

Only five previous examples of abnormal hearts in *R. temporaria* and one in *R. pipiens* have been recorded (see O'Donoghue(8)), and while the present is most like the last of these, it differs in certain respects.

Example 22 illustrates a failure of the heart to undergo normal flexure. The primitive heart tube at a very early stage is straight, but shortly afterwards when four enlargements representing the sinus venosus, atrium, ventricle and bulbus cordis make their appearance, the tube becomes bent in an **S** shape. The sinus venosus occupies the left posterior end and the bulbus cordis the anterior right end, but as development proceeds the **S** becomes rotated into the dorsi-ventral plane. Compression takes place so that the bulbus cordis is pressed down upon the atrium which by now is divided into two. The various parts of the heart in the present specimen have become adult as far as structure is concerned. It has not retained any of the developmental stages of flexure passed through by the normal heart. In the sense that it has not passed through such stages, it may be regarded as an unflexed heart but, owing to the anterior and posterior ends being relatively fixed by the arteries and veins, as pointed out above, it was forced to bend in some way in order to accommodate its increase in length.

While, as will be seen, embryology gives a key to some of the abnormalities of the blood vessels, it is also true that their study suggests problems that will require further or more detailed embryological observations for their solution.

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ABBREVIATIONS USED IN TEXT-FIGURES

A. abdominal vein; *An.* anastomosis between external jugular veins; *B.* brachial vein; *C.* cutaneous artery; *CA.* conus arteriosus; *CM.* coeliaco-mesenteric artery; *DA.* dorsal aorta; *DL.* dorso-lumbar vein; *EC.* external carotid artery; *EJ.* external jugular vein; *F.* femoral vein; *G.* genital vein; *GB.* gall bladder; *H.* hepatic vein; *I.* innominate vein; *IC.* internal carotid artery; *IJ.* internal jugular vein; *IR.* inter-renal vein; *Is.* ischiadic vein; *L.* lingual vein; *LA.* left anterior vena cava; *LAt.* left atrium; *LP.* left persistent posterior cardinal vein; *LRP.* left renal portal vein; *Lx.* larynx; *M.* mandibular vein; *MC.* musculo-cutaneous vein; *P.* pelvic vein; *PA.* pulmonary artery; *PC.* posterior vena cava; *RA.* right anterior vena cava; *RAt.* right atrium; *RP.* right persistent posterior cardinal vein; *RRP.* right renal portal vein; *S.* subclavian artery; *SA.* systemic arch; *SC.* subclavian vein; *SS.* subscapular vein; *SV.* sinus venosus; *TA.* truncus arteriosus; *V.* vesicular vein; *Ve.* ventricle.

ANATOMICAL NOTES

A NOTE ON THE FIRST LUMBAR NERVE (ANTERIOR RAMUS)

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The present writer (in collaboration with E. P. Stibbe, R. J. Gladstone and C. P. G. Wakeley) has drawn attention (1930, 1932, 1933) to the fact that the intercostal nerve is not a simple single nerve as current descriptions implied. Briefly, the ground-plan of the intercostal nerve was described as follows (fig. 1). Behind the region of the

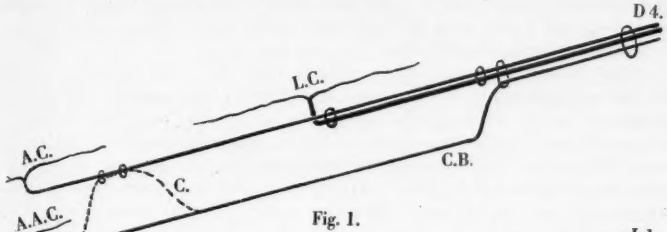

Fig. 1.

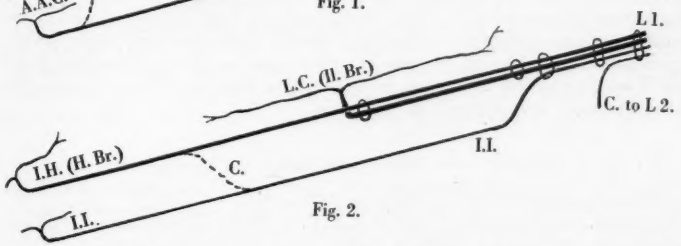

Fig. 2.

A.A.C. accessory anterior cutaneous. *A.C.* anterior cutaneous. *C.* communication between collateral and "main" nerves. *C.B.* collateral branch. *C.* to *L* 2. communication to second lumbar nerve. *D* 4. fourth intercostal nerve. *I.H.* (*H.Br.*) ilio-hypogastric nerve (hypogastric branch). *I.I.* ilio-inguinal nerve. *L.C.* lateral cutaneous. *L.C.* (*Il.Br.*) lateral cutaneous (iliac branch of ilio-hypogastric). *L*1. first lumbar nerve.

angle of the rib the intercostal nerve exists in the form of a single stem which, however, consists of three nerve trunks closely applied to each other and wrapped up in a common sheath. The terminal divisions of the intercostal nerve stem are as follows:

(1) *The "main" intercostal nerve.* This runs forwards in the upper part of the intercostal space to end as an anterior cutaneous nerve by the side of the sternum (in the case of the upper intercostal nerves) or near the lateral border of the rectus abdominis (in the case of the lower nerves).

(2) *The lateral cutaneous nerve.* This is the largest of the three divisions. For some distance in front of the angle of the rib it runs forward bound up in the same sheath as the "main" intercostal nerve. It leaves the latter nerve near the mid-axillary line and runs obliquely through the chest wall to become cutaneous.

(3) *The collateral branch of the intercostal nerve.* This leaves the "main" intercostal nerve stem at the angle of the rib and immediately runs downward to the lower

part of the intercostal space, whence it continues forward near the upper border of the lower rib to end as an accessory anterior cutaneous nerve at the lower part of the intercostal space by the side of the sternum (in the case of the upper intercostal nerves) or near the linea alba (in the case of the lower nerves). In the anterior part of its course the collateral nerve may rejoin the "main" intercostal nerve for a short distance or it may send a communicating branch upwards to join the "main" nerve in the front part of the chest wall.

The object of the present note is to draw attention to the fact that the anterior ramus of the first lumbar nerve has a similar ground-plan (fig. 2). In the posterior part of the abdominal wall it consists of a single trunk which divides into ilio-hypogastric and ilio-inguinal nerves, the former in its turn giving off a lateral (iliac) cutaneous branch, whereas the ilio-inguinal nerve is devoid of such a lateral cutaneous branch. Thus the main forward continuation (hypogastric branch) of the ilio-hypogastric nerve is comparable with the "main" intercostal nerve, the iliac branch of the ilio-hypogastric nerve with the lateral cutaneous branch of the intercostal nerve, and the ilio-inguinal nerve with the collateral branch of the intercostal nerve.

This conception may simplify teaching and will remind the student that only the "main" nerve (ilio-hypogastric) gives off a lateral cutaneous branch. It will also facilitate the understanding of the frequent failure to find separate ilio-hypogastric and ilio-inguinal nerves in the back part of the abdominal wall, the collateral (ilio-inguinal) branch of the first lumbar nerve in these cases being given off farther forward than usual. Above the first dorsal nerve and below the first lumbar nerve the spinal nerves become complicated by their participation in the prevertebral plexuses. In this connection it may be further noted that the first lumbar nerve (ilio-hypogastric and ilio-inguinal) resembles the intercostal nerves in that it passes laterally between the costal processes of the first and second lumbar vertebrae, and therefore does not pierce the psoas major muscle, whereas the other lumbar nerves descend in the formation of the lumbar plexus across the corresponding lumbar costal processes and thus pierce the "costal" attachments of the psoas.

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RETROPERITONEAL HERNIA

By N. PAN
Professor of Anatomy, Medical College, Calcutta

As cases of retroperitoneal hernia are not frequently met with I am publishing this case of hernia into the paraduodenal fossa which was dissected in the Department of Anatomy. The subject was an adult female whose abdomen, on being opened during the course of dissection, presented a large swelling below and under cover of the transverse colon and its mesocolon. The swelling (fig. 1) was fusiform in shape with the long transverse diameter almost horizontal and measuring 5 in. The greatest width of the swelling was in the middle and measured $2\frac{1}{2}$ in. It presented inferiorly a little to the right side of its centre an opening which was situated, with slight obliquity, in front of the bodies of the 4th and 3rd lumbar vertebrae. The opening was reniform, being encroached upon from behind by the vertebral column. It was bounded in front by a crescentic fold of peritoneum, along the free thickened margin of which the left colic artery with the inferior mesenteric vein passed. The left and lower end of this crescentic fold was attached to the left side of the 4th lumbar vertebra where the left colic branch of the inferior mesenteric artery, accompanied by the inferior mesenteric vein, entered it. Its right and upper end was blended with the under surface of the transverse mesocolon towards its right end. The longest oblique diameter of the opening was $2\frac{3}{4}$ in. and the anteroposterior diameter was $1\frac{1}{4}$ in. From the back of the opening a loop of the jejunum protruded, and it appears hardened *in situ* in fig. 1. In front of this was seen the emerging coil of the jejunum. The loop and the coil lay free in the opening. The covering fold of peritoneum was the *Paraduodenal fold*, containing in its free margin the left colic artery and the inferior mesenteric vein. The fossa leading from the opening was the paraduodenal fossa.

The greater curvature of the stomach lay in contact with the transverse colon, to which it was connected by the fused and shortened gastro-colic ligament. This hardly measured more than $\frac{1}{2}$ in. On dividing this short ligamentous band the postero-inferior surface of the stomach was found loosely adherent to the upper layer of the transverse mesocolon. Separation of the fused surfaces was easily effected by the fingers and the transverse mesocolon was traced to its attachment to the anterior border of the pancreas. Fig. 2 shows the structures at the floor of the fossa, exposed by incising the wall of the sac and removing the coils of the jejunum contained in the fossa. These coils measured 3 ft. in length, commencing from the duodeno-jejunal flexure, which was seen on the floor. The floor measured 5 in. in the transverse and 4 in. in the vertical diameter. It presented from right to left: (1) the third part of the duodenum which abruptly terminated on the right side of the second lumbar vertebra in the duodeno-jejunal flexure (fig. 2); (2) the bodies of the 2nd and 3rd lumbar vertebrae and part of that of the 4th, with the aorta overlying them; and (3) the lower third of the left kidney. The coils of the jejunum enclosed in the fossa were quite healthy. Those lying loose in the opening of the sac, and the healthy appearance of the enclosed gut showed that strangulation of the gut at the margin of the opening was not the cause of death. The other peritoneal fossae in the neighbourhood of the ilio-coecal junction and the sigmoid colon were normal.

The inferior mesenteric vein ascended along the free margin of the paraduodenal fold. At first it lay to the left of the left colic artery and then crossed, under cover of the artery, to its right side. Then it passed upward to open into the splenic vein near its junction with the superior mesenteric vein behind the pancreas.

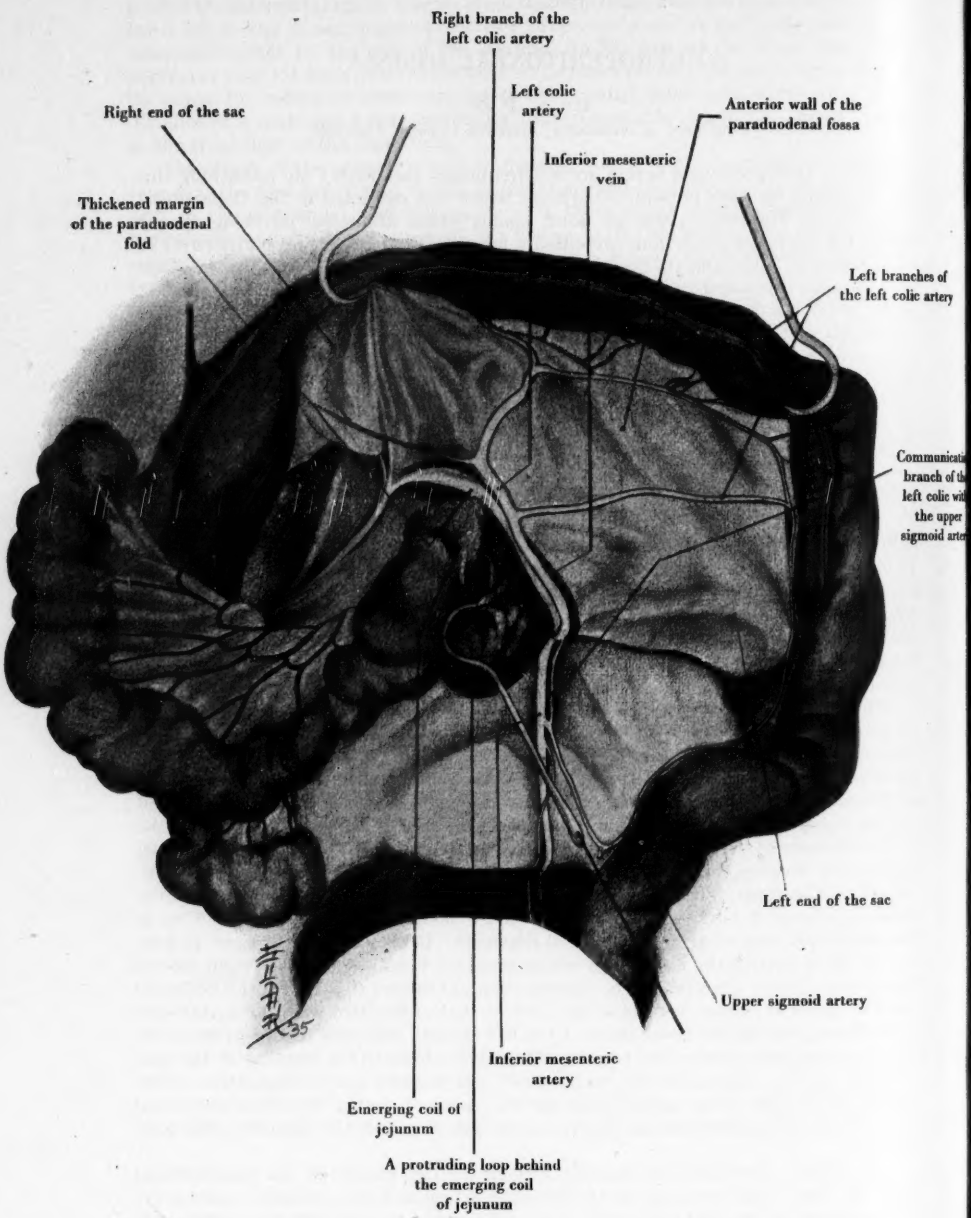

Right branch of the left colic artery
Left colic artery
Anterior wall of the paraduodenal fossa
Right end of the sac
Inferior mesenteric vein
Thickened margin of the paraduodenal fold
Left branches of the left colic artery
Communicating branch of the left colic with the upper sigmoid artery
Left end of the sac
Upper sigmoid artery
Inferior mesenteric artery
Emerging coil of jejunum
A protruding loop behind the emerging coil of jejunum

Fig. 1.

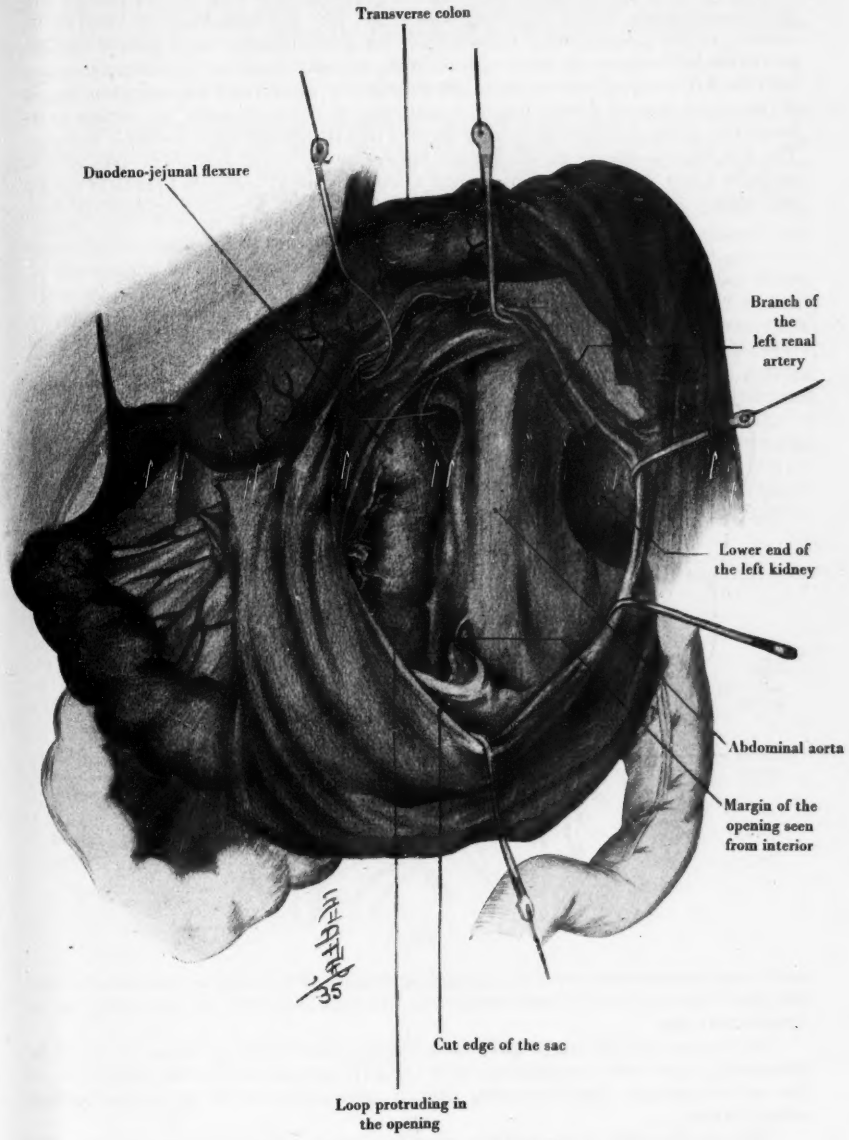

Transverse colon
Duodeno-jejunal flexure
Branch of the left renal artery
Lower end of the left kidney
Abdominal aorta
Margin of the opening seen from interior
Cut edge of the sac
Loop protruding in the opening

Fig. 2. Showing the floor of the sac.

The left colic artery, before entering the paraduodenal fold, gave a minute twig of communication to the upper sigmoid artery (fig. 2). Reaching the front of the opening of the paraduodenal fossa it gave off a left branch which passed upwards and to the left towards the proximal end of the descending colon. There it anastomosed with the left terminal branch of the left colic artery, above, and the ascending branch of the upper sigmoid artery, below. Continuing its course towards the middle of the transverse colon, the left colic artery divided into its left and right terminal branches. The left branch passed to the splenic flexure of the colon and there anastomosed with the lower left branch of the parent trunk. The right terminal branch of the left colic artery passed upwards and to the right towards the hepatic flexure of the colon

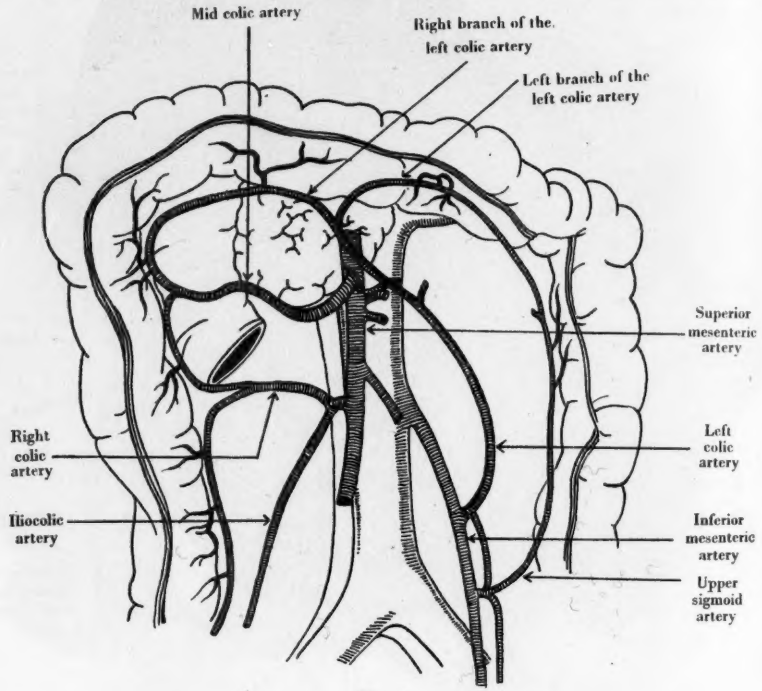


Fig. 3.

and there anastomosed with the ascending branch of the middle colic artery. Thus the distribution of the left colic artery was like that of the middle colic artery to the transverse colon.

The upper sigmoid artery gave off a long ascending branch which supplied the descending colon and anastomosed with the left branches of the left colic artery at the splenic flexure. Its descending branch anastomosed with the second sigmoid artery below.

The colic branches of the superior mesenteric artery arose from its right side. The ileo-colic and the right colic arteries had a common origin below (fig. 3). The middle colic artery came from a higher level (fig. 3), descended a little and then passed to the right where it divided into an ascending and a descending branch. The ascending branch supplied the ascending colon and anastomosed with the right branch of the

left colic artery at the hepatic flexure. The descending branch anastomosed with the ascending branch of the right colic artery. Thus, the colic branches of the superior mesenteric arteries had a limited distribution up to the hepatic flexure of the colon.

Comments

In this case the arterial distribution to the colon gives a clue to the formation of the paraduodenal fold and fossa. The superior mesenteric artery is the artery of the mid-gut and the inferior mesenteric artery is that of the hind-gut. The colic branches of the superior mesenteric artery supply the colon up to the hepatic flexure, whereas the left colic branch of the inferior mesenteric artery supplies the transverse colon from the hepatic to the splenic flexure. In the normal rotation of the U-shaped tube of the mid-gut in the third month of foetal life, the distal end of the mid-gut forms the splenic angle or flexure of the colon in the left hypochondriac region, and carries the distribution of the middle colic artery with it up to that angle. But the distal end of the U-shaped loop of the mid-gut has been fixed at the right hypochondriac region, and the proximal end of the hind-gut, which normally occupies the site of the splenic flexure, has been dragged to the right hypochondriac region, thus forming the hepatic flexure and carrying with it its arterial supply, viz. the left colic branch of the inferior mesenteric artery. Owing to this unusual dragging to the left, the left colic artery has raised the fold of peritoneum covering it to form the paraduodenal fold. The transverse colon is the proximal part of the hind-gut.

The large size of the sac, accommodating 3 ft. of the jejunum with a rather small opening, is of clinical interest; for an additional loop of the gut forced into the opening of the sac would threaten strangulation.

My thanks are due to my pupil, Sudhir Charan Bhattacharya of the third-year class, for making the drawings for me.

RECENT APPOINTMENTS

Professor D. M. Blair, to be Professor of Anatomy in the University of Glasgow.

Dr F. Davies, to be Professor of Anatomy in the University of Sheffield.

Mr E. P. Stibbe, F.R.C.S., to be Reader in Anatomy, King's College, London.

Dr W. J. Hamilton, to be Assistant Anatomist, St Thomas's Hospital Medical School.

Mr J. Whillis, M.S., F.R.C.S., to be Reader in Anatomy, Guy's Hospital Medical School.

Dr J. S. Baxter, to be Assistant Professor and Acting Chairman of the Department of Anatomy, McGill University, Montreal.

Dr G. P. Matthews, to be Lecturer in Anatomy, McGill University, Montreal.

REVIEWS

Cunningham's Manual of Practical Anatomy. 9th edition. Revised and edited by J. C. Brash and E. B. Jamieson. In three volumes. Vol. I: General Introduction; Upper Limb, Lower Limb, pp. xix + 423, figs. 193; Vol. II: Thorax and Abdomen, pp. xix + 500, figs. 238; Vol. III: Head and Neck; Brain, pp. xix + 544, figs. 240. Price 12*s.* 6*d.* net each volume.

The authors of this edition deserve well of fellow-teachers and students alike for their vivification of an erstwhile somewhat didactic text. This results from a prudent and painstaking revision, whose object has been the facilitation of the student's task of acquiring a practical conception of the intact living body, and towards which end welcome innovations have been made.

Amongst these should be cited an attractive descriptive style, improved dissection instructions, the inclusion of many new illustrations and a wealth of excellent radiographs, the replacement of the cumbrous B.N.A. by the Anatomical Society's nomenclature, the repeated reference to functional activity, and an insistence throughout upon reference to conditions in the living body, particularly as concerning visceral variations of form and position.

Recent researches have been drawn upon in the improvement of descriptive text; minutiae have been suppressed wherever possible; a nice balance of treatment permits of emphasis upon structures of major clinical importance—e.g. peripheral nerves, lymphatic vessels and glands, nasal sinuses. Specially praiseworthy is the common-sense and up-to-date treatment of such former bugbears as brain, cerebellum, peritoneum and facial musculature.

Adverse criticisms are few and trifling. The coecum is imperfectly delimited, the first intercostal nerve still lacks a lateral cutaneous branch, the subclavian artery still grooves the first rib, and the femoral sheath still derives from union of the fasciae iliaca et transversalis. But these in no way detract from the undoubted merit of a work which we confidently recommend to the student, at the same time congratulating its authors upon their excellent achievement.

Furneaux's Human Physiology. Revised edition by W. A. M. Smart, M.B., B.S. (Lond.). Nurses' Edition. (London: Longmans, Green and Co., Ltd.) 1935. Pp. viii + 348, figs. 213. Price 4*s.* net.

Dr W. A. M. Smart is to be congratulated on bringing out a completely new edition of this valuable little book. Since it was first published in 1888 it has served a very wide and useful purpose amongst members of the nursing profession and students of elementary anatomy and physiology. The work has now been thoroughly revised and brought up-to-date, and chapters added on Metabolism, Endocrines and Reproduction. The 213 well chosen and excellent illustrations add greatly to its use as a text-book either for class or home study. We can recommend this book with confidence to the student requiring an elementary introduction to the study of anatomy and physiology. It is well printed on good paper, and the price is extremely cheap.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

November 1934 *March* 1935
June 1935

RECORDED BY
R. J. GLADSTONE

EDITED BY
E. BARCLAY-SMITH

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

NOVEMBER, 1934

The Annual Meeting was held at the London School of Medicine for Women on 23 November, 1934. The President, Professor A. F. Dixon, in the Chair.

Professor R. D. LOCKHART communicated a paper on "The applied anatomy of the larynx and middle cranial fossa, demonstrated by models made of plastic wood".

Attention was drawn to the qualities of plastic wood as a modelling material, readily pliable before it sets, and capable of being carved or planed or chiselled, and then painted—in fact, treated like a piece of wood, after setting has occurred. The disadvantage of shrinking may be largely overcome by using a metal scaffolding in the building of the model.

A paper upon certain features in the Applied Anatomy of the Larynx will be submitted for publication in the *Journal of Anatomy*.

Mr F. E. NEGUS congratulated Prof. Lockhart on the excellence of the models, and remarked that the lateral fibres of the posterior crico-arytenoid muscles have a special function in separating the arytenoid cartilages. He also stated that there was a liability for laryngologists to mistake the swelling caused by the cartilages of Wrisberg for that produced by the arytenoid cartilages.

Professor LOCKHART read a paper for Dr F. W. LANCHESTER, F.R.S., on "Directional fixity and the transitory visual impression or fleeting image".

In this paper the author distinguishes between the transitory or fleeting image and the after-image, and gives the results of observations made in accordance with a definite procedure laid down, in which radical differences are shown to exist between the transitory or fleeting image as sensed when a lamp is moved across the field of vision, and when the point of regard is moved, the lamp being stationary.

It is shown that the fleeting image and the after-image, when the lamp is moved, coincide, but, when the point of regard, or centre of vision, is moved they appear in two different parts of the field of vision. The inference is drawn that the fleeting image is not formed in the retina, nor in the cortical projection of the same, but in a region beyond.

The bearing of this discovery on the question of directional fixity, i.e. the sensation experienced when the eyes are moved (a change being made in the object fixated), is discussed, and the deduction is made that there must exist a system of afferent nerves capable of conveying sensation from the oculomotor muscles to the brain. This is in agreement with Professor H. H. Woollard's conclusions as set forth in his paper entitled "The Innervation of the Ocular Muscles", *Journal of Anatomy*, vol. LXV (Jan. 1931).

The author had reached this conclusion prior to the publication of Prof. Woollard's paper, and claims that the method employed may be regarded as a new weapon of anatomical research.

The author points out that if his conclusions be admitted the accepted views on "flicker" and on the blending of colour "on a time base" (as when colours are presented in rapid succession) will require revision.

Professor C. DA COSTA read a paper on the "Paraganglia, with especial reference to the carotid body", which is published in the *Journal of Anatomy*.

The cells of the primary sympathetic cord were stated to be capable of differentiating in three directions. His results confirmed those of Dr Boyd's investigations on human material.

Professor HILL, Professor WOOLLARD and Sir ARTHUR KEITH took part in the discussion. Sir ARTHUR KEITH said that he was disappointed that no allusion had been made to the nature of the influence of the carotid body on blood pressure.

In reply to a question with reference to the existence of chromaffin cells in the carotid body Prof. DA COSTA stated that he had not found chromaffin cells in the human carotid body, but had found them in various animals.

Dr A. J. E. CAVE communicated a paper on "The evolution of the spine of the axis vertebra". The spinous process of the human axis vertebra manifests a striking variety of forms, of which the classical text-book picture represents but one—that of the relatively uncommon claw-hammer type, wherein a stout prismatic stem diverges dorsally into caudally sloping flanges, each terminated by an angulated prong prolonged almost directly caudally. This particular type, though peculiar to Man alone, is not characteristic of the vast majority of spines in any given race. It is absent from Predynastic Egyptians, Australians, Bushmen and Mediterranean stocks generally. It occurs commonly, but not invariably, in Dynastic Egyptians, divers Asiatic races and Northern Europeans. It may therefore possess the racial significance postulated by Shore (this *Journal*, vol. LXV, pt. 4, 1931; vol. LXVII, pt. 3, 1933).

To assess the morphological value of this particular form of axis spine, and to elucidate its evolutionary development, the anatomy of this process was reviewed throughout the Primate order, whence it was found possible to classify such spines into four groups, each representing a stage in the phylogeny of the human structure.

In all Primates the axis spine assists its annectant laminae in providing a stable platform for the execution of atlantal (and therefore of cranial) rotation. The spine itself is stabilised by the erector spinae musculature, principally the Mm. multifidus et semispinalis cervicis. Its configuration is but little affected by the suboccipital muscles attached to its cranial aspect. Its length, shape and size are largely the resultant of such modifying influences as the length of the neck, bodily posture, occipital contour, degree of jaw development, ocular activity, and, possibly, the development of some degree of "handedness".

Type 1, the primitive Primate type, obtains in Lemuroids (e.g. *Lemur*, *Galago*, *Chiromys*), some Hapalidae (e.g. *Cebus*, *Lagothrix*), almost all Cynomorphs (e.g. *Macacus*, *Papio*, *Cercopithecus*) and certain Anthropomorphs (*Simia* and, occasionally, *Gorilla*). A bayonet-shaped spinous process projects dorsally between, and is laterally compressed by, the twin columns of the erector spinae. This type is associated with the generalised cervical activity of the primitive pronograde Primate, expressed anatomically by a flat, vertical occipital planum, a backwardly directed foramen magnum and a snouted

cranium. In forms wherein the masticatory apparatus predominates, and a short thick-set neck results (*Simia, Gorilla, Papio*), the axis spine may become unduly lengthened like its more caudal fellows, representing some reduction of the functional plasticity of the cervical spine.

Type 2, the platform type, is again very primitive, though perhaps less so than Type 1, of which it may well be a modification. It is essentially a spine compressed cranio-caudally instead of laterally, although its cranial aspect may bear a definite median ridge. This type occurs characteristically in adult specimens of *Tarsius, Loris, Mycetes* (imperfectly) and *Hylobates*. Elsewhere it occurs only in the gorilla (frequently), chimpanzee and Man. The examination of a growth series of vertebrae reveals this type as the essential axis spine pattern for the Tarsiers, Gibbons, gorillas, chimpanzees and Man. It is thus seen to be associated with the adoption of a more or less upright posture, and in forms having the skull balanced upon instead of slung from the cervical spine by a suboccipital musculature of enhanced physiological importance. In such forms the foramen magnum is more caudally directed, the facial muzzle reduced and the occiput more globular and prominent.

Type 3 is but a further evolutionary modification of the foregoing, representing the response to the demands of postural habitus through the medium of a more differentiated erector spinae musculature. It is confined to the gorilla, chimpanzee and Man, although not invariable in any one of these forms. Anatomically it is a platform whose two sloping flanges divaricate laterally and but slightly caudally from the median cranial crest. To this crest and the rest of the cranial aspect is attached, as before, the suboccipital musculature, with little if any moulding influence upon the bone. To the caudal edge of each constituent flange are attached multifidus and semispinalis cervicis, thereby exerting a more oblique pull than in previous types.

Type 4 is a variety of axis spine encountered in Man only, though its configuration may be feebly foreshadowed in certain chimpanzees and gorillas. It is the flanged platform of Type 3 with the addition of angulated prongs at the flange extremities, and is a reflection of the more nicely differentiated dorsal musculature. Multifidus is attached to the whole length of the caudal edge of the flanges, semispinalis to the prongs themselves, which are directed more caudally than laterally. It is evident from dissection that the former muscle determines the basal structure of the spine in all types, whilst the latter muscle accounts for the bifidity of the tip and the dimensions and direction of the prongs. (For example, in the Australian and the gorilla, where this type is not encountered, these two muscles require artificial separation, whereas in the modern European manifesting this type such separation is unnecessary, the identity of the muscles being obvious.)

Whilst the four types enumerated obviously represent the several phylogenetic stages in the evolution of the classical axis spine, and are each equally obviously dependent upon the degree of differentiation of the erector spinae system, the great variability of pattern of spines in any race precludes too confident conclusions being drawn from such a survey. It is not easy to assign any functional advantage to any particular type of spine where more than one type occurs within a given group, nor is it obvious why muscles differentiated in some races should not be so in others. Possibly a visual factor comes in here, together with the business of manual skill, and enquiry along these lines is being pursued in the hope of gleaning a clearer conception of the question.

Professor FRAZER contributed a paper on the "Terminal part of the Wolffian duct", which is published in the *Journal of Anatomy*.

In reply to a question by the PRESIDENT, Professor FRAZER stated that the Wolffian duct took no part in the formation of the trigone, although there might be some remnants of the Wolffian system in the bladder wall. The Wolffian duct in women begins to disappear about half-way through the third month of foetal life.

Sir ARTHUR KEITH asked what is the factor causing migration of the duct, his own impression being that it is the mesoderm which produces these results and that a parallel case was furnished by the growth of the gubernaculum testis.

Professor FRAZER also demonstrated a case of Porencephaly, a description of which will be published in the *Journal of Anatomy*.

Mr A. C. MCMASTER made a communication on "The organ of Jacobson in *Sphenodon punctatum*", which will be submitted for publication in the *Journal of Anatomy* in conjunction with a further communication on the Accessory Bulb.

Dr R. WHEELER HAINES read a paper on "Cartilage sclerosis (metaplasia of cartilage into bone)". Series of longitudinal and transverse sections of the femur and tibia of a *Crocodilus niloticus*, soon after hatching, were stained with haemotoxylin and eosin. Each young primary trabecula contains a thick core of calcified cartilage with many hypertrophied cells. In the older trabeculae the cartilage undergoes a metaplastic change into bone. The matrix becomes bony, staining a deep purple instead of a clear blue, and some of the cells become so constricted and flattened as to resemble bone corpuscles, while many degenerate and disappear. This process of "cartilage sclerosis" has now been described in Amphibia, both in Urodeles and in Anurans, and in Reptilia (lizards). The observation here recorded in the crocodile and its confirmation in the Chelonia makes it very probable that a metaplasia of cartilage into bone is the typical mechanism for eliminating cartilage from the primary trabeculae in all early tetrapods, and that it is only the difficulty of observation that has prevented its recognition in the trabeculae of Mammals where the cartilaginous laminae are very thin.

Professor M. F. LUCAS KEENE and Dr E. HEWER demonstrated specimens of:

(1) "Nerve endings in the human foetus."
(2) "Reissner's fibre (?) in a human foetus."

MARCH, 1935

An ordinary Meeting was held at St Mary's Hospital Medical School, on Friday 1 March, 1935, the President, Professor A. F. DIXON, in the Chair.

Dr P. N. B. ODGERS showed lantern slides of wax-plate reconstruction models, illustrating the development of:

(*a*) "The venous valves",
(*b*) "The foramen secundum",
(*c*) "The septum secundum",

of the human heart. The paper is published in the *Journal of Anatomy*.

Professor FAWCETT commented on the considerable variation in the size of the venous valves in different specimens of corresponding ages. In some cases the left venous valve was difficult to distinguish, being very rudimentary.

Professor LOW concurred with Professor FAWCETT with respect to the variability in the development of the venous valves.

Dr F. DAVIES enquired whether there was any definite prolongation of sinus tissue into the septum intermedium, and asked, with reference to the work of Dr Tudor Jones on *Lepidosiren*, and on human embryos, whether he had found a direct continuity between the tissues of the sino-auricular and the auriculo-ventricular nodes? Dr ODGERS replied that he had not paid special attention to these points, and that his work had been chiefly directed to the gross anatomy of the parts concerned in the development of the valves and septa.

Professor DIXON referred to the possibility of the septum secundum being formed by the simple method of delamination.

Dr C. G. PAYTON read a paper on the "Growth of the pelvis in the madder-fed pig". The paper is published in the *Journal of Anatomy*. It was discussed by the PRESIDENT, Professor FAWCETT and other members of the Society.

Dr W. JACOBSON made a communication on the "Early development of the chick embryo". The paper will be submitted for publication in the *Journal of Anatomy*.

Dr A. F. W. HUGHES made a communication on the "Arterial development in the area vasculosa of the chick". The paper is published in the *Journal of Anatomy*.

Mr D. STEWART read a paper on "Innervation of the dental tissues", which will be submitted for publication in the *Journal of Anatomy*.

Professor FAWCETT communicated a paper on "The squamo-mastoid suture, and the independence of the squamo-zygomatic in the adult".

Professor C. J. PATTEN brought forward a preliminary report (illustrated with original lantern slides and specimens) which he had prepared in conjunction with Dr A. M. MACCONAILL, on "Skeletal remains found in a Limestone Crevice". The discovery was made during quarrying operations in Middleton Dale, Derbyshire, on 13 November, 1934; and they desired to acknowledge their thanks to the Management of Eyam Quarries, Ltd., for the facilities placed at their disposal in carrying out investigations. The sensational report which appeared in the Daily Press next morning, and which essayed that a giant skeleton, standing in the erect posture, 7 ft. in stature, with an extremely thin skull, occupied the crevice, was the outcome of an ingenious conjecture which collapsed on further inquiry. The skull, placed high up in the crevice, partly embedded in a matrix of earth and small stones, with the face exposed and looking directly forwards, belonged to an adolescent female; whilst at a lower level, also partly embedded, were found bones of the upper extremities, a few ribs, and bones of the lower extremities, in that order from above downwards, and these belonged to an adult male. It is noteworthy that the skull, the arm, and leg bones, were separated respectively by quite

thick masses of the matrix. Hence the first impression, while the parts lay *in situ*, was that of a very tall individual, really an exaggerated image! A moment later, the skull of an adult male was laid bare at a lower level, by a stroke of the pick-axe—a massive skull with a very thick vault—and now, when excavated from the matrix and compared with the thin-walled adolescent skull, it was not unnatural to conjecture that the supposed giant possessed a peculiarly thin skull! Curiously enough, the adult skeletons in this series represented individuals of short stature, averaging 5 ft. 3 in. When all material was collected and sorted, even before the fragments were pieced together, it became evident that the skeletal remains of twelve persons were represented, including adult men, women, adolescents and young children. The rock crevice measured 10 ft. high, 5 ft. deep in its lower third, 2 ft. deep in its upper two-thirds, and, on an average, 2 ft. wide. Obviously, such a limited area could not possibly accommodate twelve complete skeletons, more especially when loosely embedded in a matrix of earth and small stones forming fine rubble. It would be still more impossible to inter twelve bodies together in a space of these dimensions. The crevice is closed above by soil only 2 ft. deep. Formerly this roof may have been so much thicker that an interment could have been made therein. At a later period the crevice in all likelihood became widened by the action of water, and probably at least some of the bones in question were washed down in light subsiding soil and trapped within the limestone walls. In view of the fact that numerous bones of individuals were found widely separated, and lay embedded in a matrix of earth and in certain spots were encased in porous rubble, it seems reasonable to infer that the action of water was the main factor in producing a decided shuffling of the material. Evidently, water was able to percolate from the limestone walls through parts of the matrix freely and in sufficiently large quantities to produce copious stalactiform deposits. On the contrary, some bones, escaping the action of water, were devoid of incrustations. However, in all cases mineralisation was well developed; indeed, the skulls and large bones of the limbs in particular were so pronouncedly brittle that it was necessary to handle them with the utmost care. A complete female skull—excavated from a spot in the lower strata where the matrix was quite sodden with moisture—appeared densely clothed in a white chalky deposit. Within the cranial cavity were hand-bones, foot-bones, coccygeal vertebrae, some fragments of other vertebrae and of a few ribs belonging to a child, also a couple of sound permanent teeth, together with quantities of snail-shells. Obviously, this curious miscellany was washed through the foramen magnum, which pointed conveniently in an upward and outward direction. Analysis clearly indicated that the bones and teeth were components of different skeletons! They were well washed, a fact which suggests that they descended in the track of a subterranean current, which flowed freely through porous rubble during the rainy season. When the entire osseous collection was massed together it was soon realised that the reconstruction of the skeletons would prove difficult and tedious; albeit, it was none the less an absorbingly interesting task. Several of the skeletons were in such a fragmentary state that the closest scrutiny was called for before synthesis could be completed. The skull and a few cervical vertebrae of a sheep together with fragments of the lumbar vertebrae of a horse were also discovered, all being scattered about in the middle layer of the matrix. No doubt their proximity was accidental and would throw no light upon the cultural level of

the persons, the skeletons of whom are being investigated. Indeed, these sparse remains of domestic *herbivores* tend rather to intensify than resolve the mystery of the presence of such a remarkable array of human remains in such a curtailed space. In conclusion, the following points may be summarised in a general way, following this preliminary stage of our researches. The skeletons point to a rather primitive, rough, very muscular type, of extant Man (*Homo sapiens*). Stature short, probably reaching $5\frac{1}{2}$ ft. Bones and teeth sound; cusps of latter well worn, indicating consumption of coarse, tough food, such as raw root vegetables for the most part. Cranial capacity moderate, 1300–1400 c.c.; dolichocephalic; nose and palate characteristically narrow; flattening of tibia to some extent. On the whole, the type is markedly uniform in adults, adolescents, and children. It would seem, therefore, that these skeletons belonged to one or at most two families of a contemporaneous generation. Nevertheless, since no implements, bones of wild animals, now extinct in the British Isles, or other relics, were procured in or near the limestone crevice, it would be idle at the moment to offer any definite opinion in regard to when these persons lived, and the phase of culture to which they attained. It has been suggested, however, that they may have been victims of the terrible Eyam plague which in the year 1666 devastated the adjoining village. Perhaps some support might be given to this idea in view of the fact that the direful exigencies of the situation forced some burials to be made with all possible haste in odd spots outside the precincts of cemeteries. But against such a theory we have to take into account geological changes, the somewhat primitive characters and definite mineralised state of the bones; also the short stature, computed by investigation on the reconstructed skeletons. These, and many other features which cannot be cited in this preliminary report, connote a type of people who probably lived at a period considerably earlier than 1666.

Miss BARRY demonstrated a "Dissection of the abdominal sympathetic system in a one-year-old child".

JUNE, 1935

The Summer Meeting was held at Trinity College, Dublin, on 7 and 8 June, 1935, the PRESIDENT, Professor DIXON, being in the Chair.

Professor A. B. APPLETON read a paper on "The form of the pleural cavities in the cadaver".

Professor HARROWER and Professor WOOLLARD commented on certain changes in form being produced by distortion, and Professor HARROWER remarked that the cast of the phrenic nerve appeared to be unduly large.

Professor H. H. WOOLLARD contributed a paper on "Cutaneous nerve-endings", which will be published in the *Journal of Anatomy*.

Dr W. O'CONNELL and Professor H. H. WOOLLARD made a communication on "Intra-neural plexuses".

Dr H. GRAY and Professor H. H. WOOLLARD communicated a paper entitled "Observations on lymphatics with remarks on technique", which will be submitted for publication in the *Journal of Anatomy*.

Professor E. J. K. Ewatt read a paper on the "Early stages of development of the lymphatic system", which will be submitted for publication in the *Journal of Anatomy*.

Dr W. J. Hamilton showed lantern slides illustrating the "Early development of the mesoderm and notochord in the ferret". He showed that the mesodermal cells at first arise from the ectoderm of the embryonic disc at the site of the posterior end of the future primitive streak. The primitive streak differentiates from behind forwards and, in the ferret, is a relatively long structure; it may be half the length or more of the embryonic disc at the early stages. The mesodermal cells grow outwards and forwards as two sheets from the sides of the primitive streak. The apices of the two sheets of the mesoderm eventually meet at the front end of the embryonic disc. Henson's knot does not appear until the primitive streak is well developed. In all the specimens examined the knot is separated by a narrow neck from the anterior end of the primitive streak. Few or no mesodermal cells arise from the sides of Henson's knot. From the anterior part of the knot the archenteric process or head process is developed. A well-developed but short archenteric canal is present in the archenteric process. This canal opens above into the amniotic cavity via the blastopore and below into the yolk sac. The cells of the roof of the archenteric process become intercalated in the yolk-sac endoderm and form the archenteric or notochordal plate. The archenteric plate ends anteriorly in the prochordal plate of thickened endoderm.

Professor Bryce remarked that the development of the ferret was special, and that in his opinion one could not generalise concerning the origin of the mesoderm in the human subject and Mammals generally from the conditions present in the ferret.

Dr Y. Appajee gave a demonstration on "Some peculiarities in the histology of the brain of the hedgehog with lantern slides of the drawings made of sections under the Eddinger apparatus". The points upon which he laid stress were:

(1) That the olfactory brain of this animal showed great enlargement in its component parts, as was previously shown by Watson, Elliot Smith, and Le Gros Clark. It has massive olfactory bulbs, equally large olfactory tubercles and extensive piriform cortex. The rhinal fissure is very pronounced and is situated dorsolaterally on the hemisphere.

(2) The so-called anterior olfactory nucleus (Herrick, 1924) appears on the dorsal aspect and not on the lateral side of the ventricle as in other Mammals. Also it shows greater differentiation by presenting three cellular layers instead of two zones as in other animals, the exact significance of which is not known.

(3) The accessory olfactory bulb is present and is the first relay station for the nerve of Jacobson. It has a distinct anterior olfactory nucleus of its own.

(4) The primitive marsupial stage in the composition of its ventral commissure can be demonstrated, a detailed description of which will be submitted for publication in the *Journal of Anatomy*.

(5) It has a massive amygdaloid nucleus, and is highly complex. Though in the main it shows the various divisions of Johnston, it shows considerable individuality and variations as to details.

(6) The habenulo-peduncular and the mammillo-thalamic tracts run together for a considerable distance in the di-encephalon, and consequently in a

transverse section of the latter, the three landmarks of cross-sections of habenulo-peduncular, mammilo-thalamic tracts and the fornix bundle are not obvious as is commonly the case in other Mammals.

Professor WOOLLARD remarked that the lantern slides shown by Dr Appajee seemed to disclose with unusual clarity the structure of the amygdaloid nucleus, and this animal might afford favourable material for the study of this nucleus.

Professor LOCKHART remarked that Dr Appajee was working in his department and that they would like an opinion as to whether uni- or bi-polar stimulation of the cortex was the more suitable.

Professor BEATTIE replied that in his opinion bi-polar stimulation of the cortex was the better.

Dr J. BEATTIE and Dr A. S. KERR, read a paper on "Some observations on the hypothalamus", of which the following is a brief abstract. The advantages of physiological experiments in localising functions to anatomical regions of the hypothalamus were discussed. A series of observations on the effect of electrical stimulation of the hypothalamus on the tonus and activity of the urinary bladder was described. The exact point of stimulation was controlled by histological sections. Centres were shown to exist in the anterior hypothalamus and tuber cinereal region for increase of bladder tonus, and in the posterior hypothalamus for its decrease. Evidence for the existence of fibre tracts descending from these nuclei through the mid-brain was also brought forward.

Professor WOOLLARD asked whether the results obtained were dependent on stimulation or destruction? For instance, Dutton and Watts were inclined to explain their cognate results in terms of release. Moreover, the lesions exhibited did not seem to bear any very precise relationship to hitherto described nuclei.

Professor BEATTIE replied that their effects were immediate, and were in their opinion due to stimulation. He thought the anatomical criteria of what was a nucleus were often subjective. Their lesions had given them evidence of descending degeneration, showing sympathetic tracts lateral to the aqueduct, and parasympathetic tracts ventral to the aqueduct.

Dr F. H. BENTLEY made a communication on "Studies in wound healing by tissue culture methods". Small squares of mammalian embryo skin upon which superficial wounds have been inflicted were cultivated *in vitro*. Regeneration of epithelium and connective tissue took place, and by fixing and sectioning the explants at different stages the process of healing could be followed. The results suggest that actively growing epithelium produced an alteration in activity and arrangement of the subjacent fibrous tissue. These changes were studied further by grafting skin on explants of connective tissue *in vitro*. Orientation of the fibroblasts and collagen fibrils in lines converging on the new growing epithelium took place, together with increased collagen fibril formation. The changes were not seen in relation to actively growing renal or glandular epithelium, and were considered to be a specific effect of skin epithelium.

Professor WOOLLARD asked if the peculiar reaction of the dermal tissue was obtained with epithelium of mucous membrane. He suggested that investigation of electrolytes, especially cations released from cells, might be of interest. These had been shown to affect intra-epithelial nerve-endings. In-

cidentally the experiments performed proved that there was no formative influence of fibrous tissue on renal epithelium as had been suggested formerly.

Dr W. HARPER contributed a paper on:
(1) "Vascularity of the cardiac valves".
(2) "Blood cysts on the cardiac valves".
The paper is to be submitted for publication in the *Journal of Anatomy*.

Dr R. J. GLADSTONE showed a series of lantern slides demonstrating the "Occurrence in a human embryo* of 19 mm. C.R. length, of the following developmental defects:

(1) A caecal termination of the paratracheal segment of the oesophagus.

(2) A tracheo-oesophageal fistula. The upper patent end of the distal segment of the oesophagus, joined the dorsal aspect of the trachea, just above its bifurcation.

(3) Diminution in size and stenosis of the oesophagus in the upper part of the posterior mediastinum. In this segment the lining epithelium and muscular coat were present but imperfectly developed.

(4) Defective development of the cardiovascular system, including persistence of the distal part of the right dorsal aorta, which terminated as the right subclavian artery after having given off the vertebral, highest intercostal, and internal mammary arteries. The right common carotid artery arose directly from the arch of the aorta, and the right inferior laryngeal nerve pursued a direct course to the larynx, trachea and upper segment of the oesophagus.

(5) Absence of the posterior nares; the lateral walls of the nasal cavities, being blended posteriorly with the septum.

(6) Abnormal development of the occipito-cervical region of the skull and vertebral column.

(7) Abnormalities in the development of the brain.

(8) Defective histogenesis."

In discussing the nature of these defects he alluded to other cases in which the association of a distal origin of the right subclavian artery, with a tracheo-oesophageal fistula has been described; more particularly those of Sir Arthur Keith, in which an abnormal origin of the right subclavian artery was present in three out of four instances of tracheo-oesophageal fistula.

He also showed a drawing of a linear reconstruction which he made in 1915 from a normal human embryo 17 m.m. C.R. length, illustrating the period at which the distal part of the right dorsal aorta normally becomes obliterated. The lumen in this specimen had already disappeared in part of its extent, while the fourth right aortic arch and the part of the right dorsal aorta between the fourth and the last aortic arch had enlarged to form the commencement of the right subclavian artery.† This specimen corresponds very closely to a similar stage, reconstructed from a 16 mm. human embryo, and published in the series described by Congdon in 1922.‡ The two specimens considered together may be regarded as indicating that the stage at which the definitive origin of the right subclavian artery is determined is one at which the embryo has attained a length of approximately 16–17 mm. In normal human embryos of 19 mm. C.R. length, namely the stage of development corresponding to that of the

* The author is indebted to Professor J. E. Frazer for his kind permission to make use of this specimen.

† *J. Anat.*, Lond., vol. XLIX, p. 362, 1915.

‡ *Contr. Embr. Carnegie Inst. Wash.* No. 61, 1922.

specimen of tracheo-oesophageal fistula described, the distal part of the right dorsal aorta has usually completely disappeared.

After discussing the various views which have been put forward in explanation of the occurrence of tracheo-oesophageal fistula, and of its occasional association with a direct origin of the right subclavian artery from the aorta, he stated that in view of the multiplicity and extent of the developmental defects in this specimen, and to the fact that in the majority of cases of direct origin of the right subclavian artery from the aorta this anomaly was unaccompanied by tracheo-oesophageal fistula, the evidence appeared to be in favour of the anomalies being primarily due to some general cause, e.g. a diseased condition of the chorion and embryo interfering with normal development, rather than to a local cause such as pressure of a persistent right dorsal aorta on the oesophagus and trachea. The evidence that in some cases of tracheo-oesophageal fistula a considerable length of the oesophagus is stenosed, or is reduced to a fibrous cord below the site of the fistula, and that in other cases atresia duodeni, atresia ani and other occlusions of the alimentary tract have been present in the same individual is also opposed to the view that the pressure exerted by a persistent right dorsal aorta is the primary cause of the interruption in the continuity of the oesophagus and of the fistula.

In the specimen described there is definite evidence of impaired nutrition and a diseased condition of the tissues, affecting more particularly the muscular and endothelial walls of the heart and blood vessels, and also affecting the muscular tissue and endodermal lining of the alimentary and respiratory tracts. As a result of these changes, the usual sequence of developmental changes may be interrupted, and vessels which normally persist may become occluded, e.g. the fourth right aortic arch, while others which normally disappear completely may persist in order to carry on the circulation. Similar occlusions and irregularities of development may occur in the alimentary and respiratory tracts leading to stenoses, atresias or fistulae in various situations. The coexistence of various anomalies in different parts of the body may thus be readily explained, while their localization in certain situations more frequently than in others is probably determined by local conditions such as varying degrees of traction and pressure occurring in special parts at different stages in the growth of the embryo.

The author hopes, at a later date, to submit an account of the anomalies of the brain and occipito-cervical region for publication in the *Journal of Anatomy*.

Professor C. M. West gave an account of the study of a human embryo of 25 somites, which will be submitted for publication in the *Journal of Anatomy*. He also exhibited a set of radiograms of the complete skeleton of a foetal whale, *Pseudorca crassidens*, which will be submitted, together with an account of the specimen, for publication in the *Journal of Anatomy*.

Dr Alice Carleton read a communication on "The limb bones of the extinct lemurs of Madagascar", of which the following is a summary:

(1) The limb bones of *Archaeolemur* and *Lemur* were contrasted, and it was shown that they closely resemble one another in proportion and in detail but that those of *Archaeolemur* give evidence of a much greater degree of mobility.

(2) It was suggested that a number of limb bones hitherto attributed to other extinct lemurine types or to a sloth-like animal really belong to *Palaeopropithecus*.

(3) The sloth-like character of these bones is due to a convergent evolution, such as has already been noted in the slow lemurs. In *Palaeopropithecus* it has been carried even further in the extremities which were apparently used as hooks.

(4) In the relative proportions of the limbs, and in many details of the long bones, a convergent evolution was also noted with the gorilla, whose slow and deliberate gait was probably similar.

Dr E. Sheldon Friel read a paper on "Variations in the position of the transverse palatal rugae in relation to the teeth".

Dr R. Walmsley contributed a paper on the "Sheath of the rectus abdominis muscle", which will be submitted for publication in the *Journal of Anatomy*.

Dr Cave drew attention to the fact that the pyramidalis muscle was more inconstant in the Anthropoid Apes than in Man. In reply to Professor Appleton, who suggested that the absence of tendinous intersections in the lower part of the rectus was closely connected with the absence of the lower part of the posterior sheath wall Dr Walmsley drew attention to the fact that in the chimpanzee, where there was an absence of the intersections, the posterior wall still remained intact, as the transverse muscle did not come forward into the superficial wall of the sheath as it does in Man. He had investigated this question of the relations of the intersections and the absence of the lower part of the posterior wall of the sheath, and found no relationship between them.

Dr Falkiner showed specimens of human endometrium in various stages of proliferation and secretion with corresponding specimens of corpora lutea. In addition he showed specimens of early human placentation. A series of lantern slides was also shown illustrating, amongst other points, the secretory activity of the human uterine mucosa in early pregnancy and the nature of the trophoblastic invasion of the maternal vessels shortly after imbedding of the human ovum.

Dr T. Nicol read a paper contributed by himself in collaboration with Professor T. H. Bryce on "Intravitam staining of the reproductive organs in Cavia and the results of hormone experiments". The main results brought out by Dr Nicol's memoir were: (1) Special cells showing maximum amount of trypan blue occur in the endometrium only in large numbers at certain stages of the sexual cycle, and are practically absent in sexually immature animals. (2) This phenomenon, absent in ovariectomised animals, was produced by oestrin injections, but not by administration of corpus luteum hormone or anterior pituitary. (3) Fat deposit also appeared cyclic and was produced artificially in ovariectomised animals by injection of corpus luteum extract, after sensitisation of the uterus with oestrin. (4) In the pregnant animals, around the embryo, cells, proved endometrial by development, absorb the dye and form embryotrophe. The results as a whole indicate that the function of cells absorbing the dye is histiotrophic. This opens the larger biological question as to the part played by the cells of the reticulo-endothelial system in tissue nutrition, and the paper gives data to support the view that this is not merely a simple process of absorption from the body fluids, but is of a much more elaborate and complex nature and is subserved by a special class of cells, the histiotrophes.

Several speakers took part in the discussion. Dr Falkiner (Dublin) said that the results were of practical importance to gynaecologists.

Professor HARROWER (Singapore) suggested that the production of fat in the uterine epithelium by Progestin was probably of a degenerative nature.

Dr NICOL replied that the fat was more likely to be constructive in view of the stage in the cycle at which the corpus luteum normally exerted its influence, and its presence was probably related to providing nourishment for a fertilised ovum.

Professor T. H. BRYCE communicated a joint paper by himself and Dr T. NICOL, on the "Nature and development of vitally staining tissue in the pregnant uterus". The paper will be submitted for publication in the *Journal of Anatomy*.

In reply to questions asked in the discussion which followed the reading of the paper, Professor BRYCE explained that his results were opposed to those of other observers. The tissue generally regarded as ectoplacental and foetal in origin he believed he had proved to be maternal. He believed that the histiocytes prepared the embryotrophe from maternal tissue, and that this diffused into the embryo, and was thus used.

Dr R. H. HUNTER read a paper on the "Development of the prepuce", which is published in the *Journal of Anatomy*.

Dr MORRIN described a case of pseudo-hermaphroditism.

Dr F. DAVIES communicated a joint paper by Professor BLAIR, Dr BACSICH and himself on the cells of the "Posterior root ganglia", which is published in the *Journal of Anatomy*.

Professor WOOLLARD stated that he thought Ransom was right in believing that the darkly stained cells were more susceptible to reagents than the pale cells, and suggested that in the use of 3 per cent. saline solution, "class two cells" had become "class three cells"; that "class three cells" had disappeared, and that these had therefore shown an increased susceptibility. Dr DAVIES replied that hypertonic saline treatment damaged all the cells, as evidenced by the absence of the Golgi-reticulum. Normally the dark cells exhibited a well-defined Golgi network.

Dr BACSICH stated that whereas we did not know the function of the Golgi apparatus or of the tigroid-bodies it was generally accepted that these elements were features of normal healthy cells.

Professor BEATTIE complimented the authors on their work and said that he was glad to know that the dark cells were unipolar not multipolar as in sympathetic ganglia.

The PRESIDENT said that he was convinced by the demonstration of the existence of three types of cell histologically different.

Dr A. J. E. CAVE read a paper on the "Morphology of the last dorsal transverse process", which will be submitted for publication in the *Journal of Anatomy*.

Mr FITZSIMONS read notes on a case of "Plication of the small intestine within a single Mesentery". A male, aged forty years, came under his care with a history of gradually increasing vomiting for a period of six weeks. Previous history was without incident. He showed films revealing marked obstruction in the third stage of the duodenum. A drawing of the operative findings, revealing a remarkable condition, was exhibited. The abdominal

cavity was irregular in shape and lined by a thickened peritoneum. Within this cavity, and running from above downwards and to the right, was a tube-like structure about the thickness of a motor cycle tyre and about 22 in. in length. No solid viscus, liver, stomach or spleen was in this cavity. He stated that the tubular structure contained the plicated small bowel from the duodenum to the caecum, and demonstrated this on the removed specimen. He further stated that the other viscera were found, at post mortem, lying, in areola tissue, outside the cavity. Micro-photographs of sections of the bowel with its thickened peritoneal covering were also shown. He was unable to find peritoneum between the plicated loops. Mr FITZSIMONS stated that he had failed to find any recorded case which agreed with his findings. A case described by Haymond and Dragstedt, in the *Archives of Surgery*, December, 1934, was the only case having any resemblance to his.

THE TOPOGRAPHY AND HOMOLOGIES OF THE HYPOTHALAMIC NUCLEI IN MAN[1]

By W. E. Le GROS CLARK

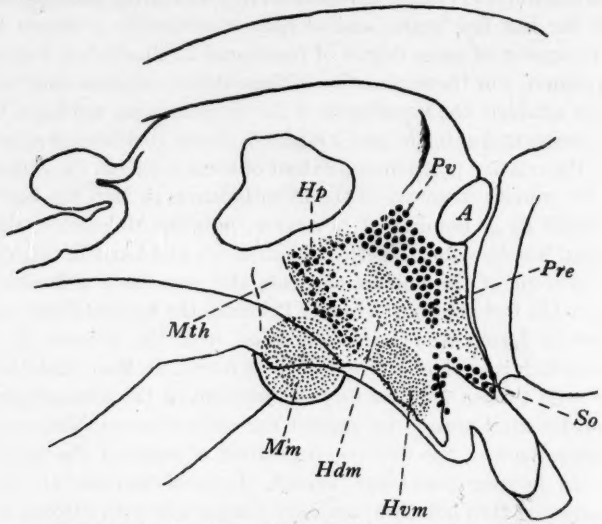
Department of Human Anatomy, Oxford

The experimental study of the mammalian hypothalamus has progressed very rapidly in the last few years, and already considerable evidence has been adduced in support of some degree of functional localisation in this region of the diencephalon. For the application of these data to clinical observation it is necessary to establish the homologies of the hypothalamic nuclei in Man with those of experimental animals, and it is also desirable to delineate as accurately as possible the relative position and extent of these nuclei in the human brain. Although the minute structure of the hypothalamus in Man has been studied in some detail by a number of observers (notably Malone (15), Greving (7), Nicolesco and Nicolesco (16), Gagel (6), Grünthal (10), and Laruelle (13)), the homologisation of some of the nuclear elements still remains a difficulty. This is partly due to the fact that in the higher Primates the hypothalamic region has been relatively foreshortened in association with the flexure of the basicranial axis which is so characteristic of these forms. In Man, with the deepening of the sella turcica and the forward rotation of the hypophysis, the infundibulum becomes pressed up against the optic chiasma (Tilney (21)). As a result of these factors, the relative disposition of some of the hypothalamic elements has become somewhat altered. Coronal sections of the human hypothalamus are thus not always readily comparable with coronal sections of the hypothalamus in lower Mammals. For the purpose of this study, serial sagittal sections have been made of the hypothalamus and compared with a series of coronal sections. In addition, numerous observations have been made on the structure of the hypothalamus in lower Mammals, especially the lower Primates and the Insectivores.

As with other parts of the brain, comparative studies of the hypothalamus are made difficult by the terminological confusion which still prevails. This confusion has been introduced by the application of different names by different authors to the same or homologous cell groups. One of the objects of this paper is to attempt some uniformity in nomenclature, and especially to bring into line the descriptive accounts of the human hypothalamus with the extensive comparative studies which have recently been made of this region (principally by American neurologists). Sagittal sections of the hypothalamus close to the

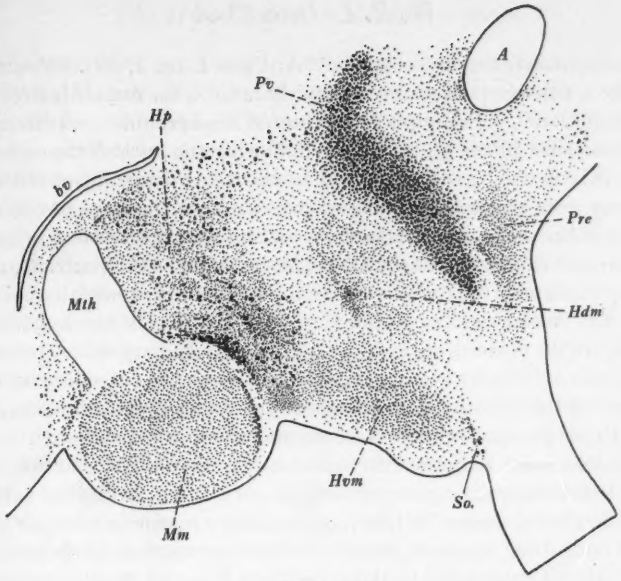
[1] The material of this paper formed part of a communication made at the International Neurological Congress held in London in August 1935.

median plane show that this region can be conveniently subdivided into four separate areas. These areas are already distinct during the fourth month of foetal development when, indeed, they are rather better demarcated than in the adult (Plate II, fig. 1). Rostrally is the pars supraoptica of the hypothalamus which contains two of the best defined of all the hypothalamic nuclei, the paraventricular nucleus and the supra-optic nucleus. Behind the optic chiasma is the pars infundibularis of the hypothalamus containing the ventro-medial and dorso-medial hypothalamic nuclei close to the mid-line and, more laterally, the lateral hypothalamic area. More caudally is the retro-infundibular part of the hypothalamus containing the posterior hypothalamic

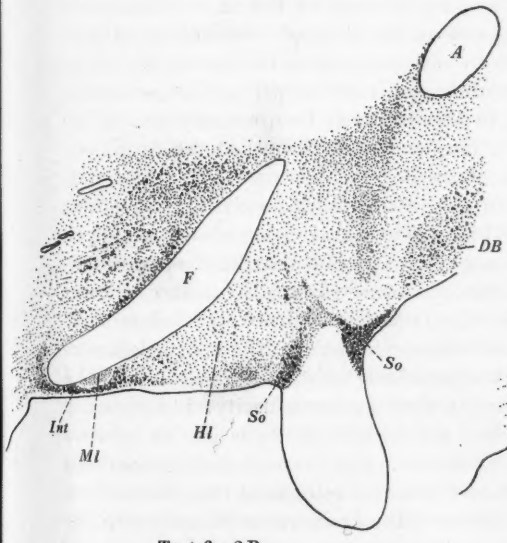


Text-fig. 1. A diagram showing the hypothalamic nuclei projected on to the lateral wall of the third ventricle. This diagram has been made by taking a tracing from a photograph of the brain which was afterwards serially sectioned in a sagittal plane. The extent of the nuclei was reconstructed from the serial sections. $\times 2\frac{1}{2}$.

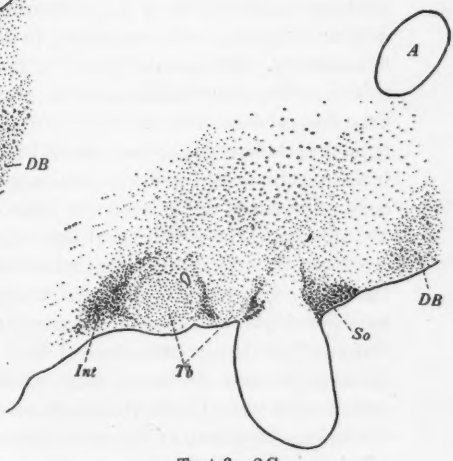
nucleus which is interposed between the ventro-medial hypothalamic nucleus in front and the mammillary body behind, and which extends upwards and backwards to become continuous with the tegmental region of the mid-brain. Lastly, the caudal extremity of the hypothalamus is formed by the pars mammillaris hypothalami which contains the large medial mammillary nucleus, the small lateral mammillary nucleus, and the nucleus intercalatus. Anteriorly the hypothalamus passes without any sharp demarcation into the pre-optic area, a part of the telencephalon medium which lies ventral to the anterior commissure. Posteriorly it is bounded by postero-median ganglionic vessels which, arising from the commencement of the posterior cerebral artery, course upwards and forwards to reach the thalamus proper (text-fig. 2A, *bv*).



Text-fig. 2 A.



Text-fig. 2 B.



Text-fig. 2 C.

Text-fig. 2. A. Sagittal section of the hypothalamus near the mid-line. B. Sagittal section of the hypothalamus in the plane of the descending column of the fornix. Note the small cell groups linking up the two parts of the supra-optic nucleus. C. Sagittal section of the hypothalamus near its lateral margin. $\times 5\frac{1}{4}$.

Nucleus paraventricularis (text-fig. 2A; Plate I, fig. 1, *Pv*). This nucleus is formed by a densely packed mass of fairly large cells, rounded, oval or subtriangular in shape, with peripherally situated Nissl granules and often with an eccentric nucleus[1] (Plate II, fig. 4). By following the nucleus through sagittal sections, it is found to reach forward to the ventral margin of the anterior commissure near the mid-line; above, it extends to the level of the anterior extremity of the sulcus hypothalamicus, and below it reaches to a point 3 mm. above the optic chiasma (text-fig. 1). As projected on to the ventricular surface of the hypothalamus, the nucleus is seen to extend further caudally in its upper part, so that it here overhangs the pars infundibularis of the hypothalamus. Moreover, at its postero-superior margin it is linked up with the posterior hypothalamic nucleus by an irregular chain of large cells extending back at the dorsal limit of the hypothalamus alongside the sulcus hypothalamicus. Below, small scattered groups of large cells serve similarly to connect it with the supra-optic nucleus (as seen to better effect in coronal sections). The whole paraventricular nucleus measures 7·5 mm. in vertical and 4·5 mm. in sagittal extent. At its maximum development in transverse sections it reaches a width of approximately 1 mm. It is apparent, therefore, that the nucleus forms a broad flat plate of cells closely applied to the ependymal lining of the third ventricle.

Comparative studies show that the paraventricular nucleus is remarkably constant in its relative size and position throughout the mammalian series (compare fig. 85 in Loo's monograph on the brain of the opossum (14) with text-fig. 1 of this paper). It has been variously termed the nucleus filiformis, nucleus magnocellularis hypothalami, and nucleus periventricularis. The detailed histology of the nucleus in Mammals has been recently studied by Scharrer (19). He remarks upon the frequency of multinucleated cells in both this and the supra-optic nucleus. According to his observations these cells arise from the amitotic division of uninucleated cells, and active division of this type may be continued into adult life in Man. Scharrer has also reported cell inclusions of colloid material (staining red with van Gieson) in the paraventricular and supra-optic nuclei in Man and other Mammals (as also in lower Vertebrates). He believes that this colloid provides evidence of an independent secretory activity of the cells themselves, and he includes the two nuclei under the term "Zwischenhirndrüse". Another characteristic feature shared by the paraventricular and supra-optic nuclei is their great vascularity. It is generally assumed that this is indicative of some special functional relationship between these nuclei and the blood vascular system. But the vascularity is perhaps merely related to their cell density, for it is well recognised that the richness of the capillary bed in the grey matter of the brain varies directly with the cellular richness.

Nucleus supraopticus (text-fig. 2B and C; Plate I, fig. 2, *So*). This nucleus consists of a closely packed group of cells which straddles across the rostral

[1] These peculiar characters of the cells may readily be mistaken by the unwary for degenerative changes associated with chromatolysis.

part of the optic tract, extending from the lateral margin of the optic chiasma back to a level one-third of the distance between the caudal margin of the chiasma and the rostral border of the mammillary body. In sagittal sections it appears as a sharply circumscribed triangular group of cells immediately antero-lateral to the optic tract and a less dense group immediately postero-medial. These groups correspond to the latero-dorsal and ventro-medial supra-optic nuclei respectively of Gagel (6). The latter element extends nearer the mid-line anteriorly, reaching on to the anterior aspect of the tuber cinereum immediately behind the chiasma. In coronal sections it is not easy to see the connection between these two parts of the supra-optic nucleus, and by some authors the ventro-medial element has been regarded as a separate nuclear entity termed the nucleus pedamenti lateralis. Sagittal sections demonstrate clearly enough, however, that they are linked together by isolated groups of cells of a similar type which cross over the dorsal aspect of the optic tract (text-fig. 2 A). These connecting groups represent the pars dorso-medialis of the supra-optic nucleus of Greving (7), or the nucleus supraopticus intermedius of Gagel (6). In coronal sections the main part of the nucleus, that is to say, the latero-dorsal element, is seen as a dense clump of cells at the lateral border of the optic tract about 1 mm. in vertical and transverse diameters.

The supra-optic nucleus is present in all mammalian brains which have been examined for it, and is very constant in position and relative size. It has been variously termed the nucleus tangentialis and the ganglion opticum basale. As in Man, the medio-ventral portion in lower Mammals reaches towards or even across the mid-line immediately behind the chiasma, here extending down for a short distance into the anterior margin of the tuber cinereum. According to Spiegel and Zweig (20) the nucleus is more diffuse in marsupials, and its cells are partly strewn among the fibres of the medial fore-brain bundle. It has already been mentioned that the supra-optic and paraventricular nuclei have several features in common, e.g. cell type, secretory properties, and vascularity. They are, moreover, linked together by an interrupted chain of small cell groups extending in a dorso-medial direction and, according to Greving, by a fibre tract. Both the paraventricular and supra-optic nuclei are also believed to be in direct or indirect fibre relation with the hypophysis (Pines (17), Greving (7), Laruelle (13)). The evidence for the existence of a supra-optico-hypophyseal tract is particularly strong, being based not only on direct study of silver preparations but also on experimental degeneration. It seems probable, on the grounds cited above, that the two nuclei are differentiated elements of one functional mechanism. Comparative studies indicate, indeed, that they are represented in lower vertebrates by one common mass of cells—the nucleus praeopticus—which extends from the optic chiasma below to the wall of the pre-optic recess of the third ventricle above.

Nucleus hypothalamicus ventro-medialis (text-fig. 2 A; Plate I, fig. 1, *Hvm*). The medial part of the tuber cinereum—that part lying between the ependymal lining of the third ventricle and the descending column of the fornix—is filled

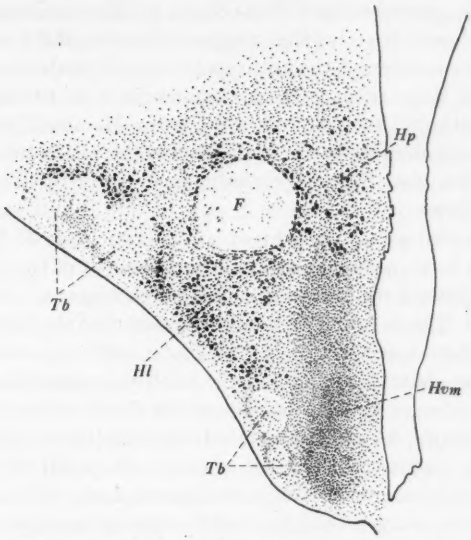
with closely packed, small, rounded or oval cells with poorly staining cytoplasm. This collection of cells has generally been included under the general term of central or periventricular grey matter. In most Mammals, however, it can readily be subdivided into dorsal and ventral condensations which merit definition as the dorso-medial and ventro-medial hypothalamic nuclei. This distinction is also apparent in Man. The ventro-medial nucleus corresponds to the nucleus principalis of the hypothalamus of Cajal (2), and to the nucleus infundibularis medialis, nucleus ovalis hypothalami, or the ventral nucleus of the tuber cinereum of some other authors. It lies between the supra-optic nucleus in front and the posterior hypothalamic nucleus behind, from both of which it is clearly differentiated in its cyto-architecture. The nucleus measures approximately 4 mm. in sagittal diameter and 3 mm. in vertical extent. In coronal sections it is seen to extend from the ventricular cavity laterally for 2·5–3 mm. This nucleus bears the closest topographical relation to the pituitary gland. According to Cajal it forms the major part of the tuberal portion of the hypothalamus in new-born Rodent material. This is confirmed by Gurdjian (11), who also implies that it becomes relatively smaller in adults. In view of these observations it is interesting to note that the ventro-medial hypothalamic nucleus is remarkably distinct and circumscribed in the human foetus. A sagittal section of the brain of a foetus of 3½ months is shown in Plate II, fig. 1, in which the nucleus appears as a compact oval mass of cells sharply outlined by a clear zone. Its appearance in coronal section in a 5 months foetus is shown in Plate II, fig. 2, where it is seen to be equally well defined. In later development, the nucleus loses its sharp and even contour.

Nucleus hypothalamicus dorso-medialis (text-fig. 2A and Plate I, fig. 1, *Hdm*). This nucleus is far less well defined, and its component cells less compactly arranged. It also lies up against the ventricular cavity, extending dorsally to the level of the sulcus hypothalamicus. Sagittal serial sections show that rostrally it runs into continuity with the medial pre-optic nucleus. It has been included by some authors under the terms nucleus infundibularis anterior and the dorsal nucleus of the tuber cinereum.

Lateral hypothalamic area. The lateral part of the tuber cinereum is somewhat complex cyto-architecturally and is therefore not easy to analyse anatomically. It occupies the same topographical position as the nucleus hypothalamicus lateralis which has been described in lower Mammals. This latter nucleus appears primarily to be developed as an interstitial nucleus in the course of the medial fore-brain bundle, and it is of importance for the reason that motor effects following experimental stimulation of the hypothalamus have in most cases been produced in this region. Although the medial fore-brain bundle is much reduced in Man as compared with most lower Mammals, it seems advisable for comparative purposes to apply the same terminology to the cell groups which occupy the same relative position. Because of the diffuse arrangement of its cells, however, it is perhaps more appropriate to apply the term "area" rather than "nucleus" to this region. By many recent authors

the lateral hypothalamic area in Man is included in part or in whole under the designations of tubero-mammillary nucleus, mammillo-infundibular nucleus, or tubero-mammillo-trigonal nucleus. Apart from the cumbrous nature of these terms, they do not indicate clearly the position of the cell groups, and they are not applicable to recent studies in the comparative anatomy of the hypothalamus.

In Man, the lateral hypothalamic area is situated laterally to the plane of the descending column of the fornix, and it is characterised by the small and somewhat scattered groups of large cells which pervade it. These cell groups



Text-fig. 3. Coronal section of the hypothalamus at the level of the tuber cinereum. ×8.

link up posteriorly with the nucleus intercalatus, while rostrally they are separated from the ventro-medial element of the supra-optic nucleus by an interval occupied by small cells. Laterally they extend to the lateral margin of the tuber cinereum; above they are continuous with a zone of large cells encircling the fornix column, the nucleus perifornicalis. Through the latter, the large cell groups of the lateral hypothalamic area are linked up with the scattered large cells of the posterior hypothalamic nucleus. These groups of large cells in the lateral hypothalamic area constitute a differentiation which is rather a distinctive feature of the human hypothalamus. They can be recognised in the hypothalamus of the chimpanzee (Grünthal), but they are by no means so conspicuous in this animal. In lower Mammals they can hardly be said to exist. Another distinctive character of the human hypothalamus is the development in the superficial part of the lateral hypothalamic area of spherical

and sharply circumscribed groups of small, lightly-staining cells which are termed the nuclei tuberis (text-fig. 3; Plate I, fig. 2, *Tb*). Their component cells are rather loosely packed in a clear matrix, and the whole nuclei are encapsuled by fibre laminae around which are disposed the large cells of the lateral hypothalamic area. The nuclei tuberis are arranged in two main groups, medial and lateral. The former is situated 2·5–3 mm. from the mid-line, and the latter 5–6 mm. Caudally they are bounded by the mammillary body and the nucleus intercalatus. It is not generally recognised that these nuclei are frequently raised up into small tubercles on the lateral aspect of the tuber cinereum, which are easily visible macroscopically. Indeed, in some cases these tubercles may be quite obtrusive (Plate II, fig. 3). The significance of the nuclei tuberis is not known. It seems that they are absent as differentiated elements in all lower Mammals except the anthropoid apes (Grünthal), and in the latter they are not so large or so sharply circumscribed as in the human brain. Spiegel and Zweig(20) have described in lower Mammals some diffuse cell groups which they identify as the equivalent of the nuclei tuberis, but if their interpretation is correct they are exceedingly ill-defined and quite undifferentiated in these forms.

Nucleus hypothalamicus posterior (text-fig. 2A; Plate I, fig. 1, *Hp*). This nucleus has not been recognised as a separate element of the hypothalamus in descriptive accounts of the human brain, although it has been identified in many lower Mammals. This is probably due to the fact that the cellular topography of the hypothalamus as a whole in Man has usually been studied in coronal sections, for the rostral boundary of the nucleus is somewhat irregular and, indeed, it is continued dorsally as far as the dorsal extremity of the paraventricular nucleus. As already pointed out, sagittal sections of the hypothalamus in the foetus (Plate II, fig. 1) and in the adult (text-fig. 2A) show the posterior hypothalamic nucleus to be a particularly well-defined cell group. In the adult it is characterised by a distinctive cyto-architecture (Plate II, fig. 5). In a rather dense matrix of small cells are scattered—either singly or in small groups—large oval or rounded cells which stain well, and in which the Nissl granules are concentrated peripherally and the nucleus is often eccentric in position (precisely as in the cells of the paraventricular nucleus). Below, these cells tend to arrange themselves in rows which curve upwards and backwards towards the postero-superior extremity of the hypothalamus along the course of the periventricular system of fibres. Dorsally the large cells are more irregularly arranged, but they form a zone which extends forwards at the level of the sulcus hypothalamicus to reach the upper end of the paraventricular nucleus. Rostrally the posterior hypothalamic nucleus is bounded by the dorso-medial and ventro-medial hypothalamic nuclei, while caudally it is limited by the medial mammillary nucleus and the mammillo-thalamic tract. Above and behind, its boundary is marked by the postero-median ganglionic vessels which are passing up to the thalamus proper. In vertical diameter the nucleus measures about 7 mm., and in transverse extent it reaches from the ventricular

cavity to the descending column of the fornix where it becomes continuous with the zone of large cells investing this tract—the nucleus perifornicalis. As already mentioned, the posterior hypothalamic nucleus has been described in a number of lower Mammals, though it is less well defined and less extensive than in Man. Rioch (18) has noted that in Carnivores it also contains scattered large cells but, here again, these cells are by no means conspicuous. The importance of the posterior hypothalamic nucleus is related to the fact that it appears to give rise to a great proportion (perhaps the majority) of the periventricular system of fibres (Gurdjian (11), Krieg (12), Beattie (1)) and may thus be regarded as an important efferent mechanism through which impulses from the hypothalamus as a whole are transmitted to the brain stem and spinal cord. Such motor functions are also suggested by the appearance of the nucleus in the foetal brain where it is seen very clearly to run into continuity above and behind with the motor tegmental area of the mid-brain (Plate II, fig. 1).

Medial mammillary nucleus (text-fig. 2A, *Mm*). In Man the mammillary body is made up almost entirely of the large ovoid medial mammillary nucleus which is composed of a homogeneous mass of rather small cells. This nucleus measures approximately 5 mm. in sagittal and 4 mm. in vertical and transverse diameters, and it is sharply circumscribed by a thin medullary capsule. The relatively large size of the medial mammillary nucleus and its conspicuous differentiation is a characteristic feature of the hypothalamus in the higher Primates. In the lower Primates and most other Mammals it is smaller, more irregular in contour, and has not the same sharp outline. As is well known, the nucleus is projected mainly on to the antero-ventral element of the anterior nucleus of the thalamus by the mammillo-thalamic tract (3). Indeed, in a graded series of Mammals there is, from the lower to the higher types, a progressive differentiation of the medial mammillary nucleus, the mammillo-thalamic tract and the antero-ventral nucleus, the whole system reaching the acme of its development in Man. Since it has been shown that the antero-ventral nucleus of the thalamus in its turn projects on to the length of the gyrus cinguli of the cortex (4), it seems that this system has been built up as a route through which impulses arising in connection with hypothalamic activities may be transferred to the neopallium, an integral part, therefore, of the process of telencephalisation.

Lateral mammillary nucleus (text-fig. 2B, *Ml*). In Man this nucleus is relatively quite small, and it is composed of smaller and more compactly arranged cells than the medial nucleus. Closely applied to the lateral convexity of the medial nucleus, it measures about 1·5 mm. in diameter, and is encapsuled on its lateral and ventral aspects by the nucleus intercalatus. In many lower Mammals (e.g. the rabbit and rat) this element is much larger relatively to the medial mammillary nucleus. In the higher Primates it undergoes a marked reduction. According to Grünthal (9) it is absent in *Cercopithecus*, but it is certainly present, though small, in *Macaca*.

Nucleus intercalatus (text-fig. 2B and C; Plate I, fig. 2, *Int*). As in lower

Mammals, this forms quite a conspicuous element of the mammillary complex. It is composed of large cells of the same type as those found scattered in the lateral hypothalamic area. They are closely packed together and invest the lateral mammillary nucleus on its lateral and ventral aspects. In some brains the nucleus intercalatus may form superficially a distinct ridge skirting the lateral margin of the main part of the mammillary eminence which, as stated above, is formed predominantly by the medial mammillary nucleus. Some confusion has arisen in the literature of the hypothalamus owing to the fact that the nucleus intercalatus has in some descriptions been called the lateral mammillary nucleus, while the group of cells here termed the lateral mammillary nucleus has been included in the designation of the medial mammillary nucleus. It is this confusion which led Crouch (5) to make the statement that the nucleus intercalatus is only to be found in Primates. The medial and lateral mammillary nuclei as here described are, however, quite distinct elements. It may be noted that while the medial nucleus gives rise to the mammillo-thalamic tract, the descending column of the fornix terminates mainly in the lateral nucleus and the nucleus intercalatus, as well as in the medial nucleus. Further, it is the nucleus intercalatus which gives rise to the mammillary peduncle.

In lower Mammals certain perimammillary nuclei have been described, such as the supra-mammillary and the pre-mammillary nuclei. They are sufficiently vaguely defined to render it doubtful whether they are worth a terminological differentiation. Similar condensations of cells are to be observed in relation to the medial mammillary nucleus in Man, but they hardly merit description here.

GENERAL CONSIDERATIONS

In its main features the hypothalamus of Man is seen to correspond with that of lower Mammals. However, it shows certain characteristics which are in contrast with all lower Mammals hitherto studied with the exception of the anthropoid apes and (to a lesser extent) the monkeys. These characteristics are as follows:

(1) The extensive distribution of large cells. This is seen in the lateral hypothalamic area where these cells are aggregated to form conspicuous groups, in the posterior hypothalamic nucleus (as seen to best effect in sagittal sections), and along the line of the sulcus hypothalamicus where they serve to link up the paraventricular nucleus in front with the posterior hypothalamic nucleus behind.

(2) The differentiation of the nuclei tuberis.

(3) The relative size and differentiation of the posterior hypothalamic nucleus.

(4) The relative size and differentiation of the medial mammillary nucleus, correlated with a high degree of differentiation of the mammillo-thalamic tract and the antero-ventral nucleus of the thalamus.

(5) The diminished size of the lateral mammillary nucleus.

These features are hardly consonant with the conception held by some authorities that the hypothalamus in Man is less complex and less differentiated structurally than in lower Mammals. This conception has recently been given credence by the detailed studies of Grünthal (8,9,10), who argues that there has been a progressive simplification of the cyto-architecture of the hypothalamus in the evolutionary scale of Mammals. He has arrived at this conclusion by counting the "nuclei" in the hypothalamus of various Mammals and comparing the results. Many of these "nuclei" are so indistinct, however, that it is unlikely that two observers will agree on the number of nuclei to be catalogued. Thus, in the dog Grünthal defines 12 nuclei, while in the same animal Rioch (18) enumerates as many as 21. Moreover, a study of Grünthal's excellent microphotographs shows, for instance, that in the human hypothalamus there are numbers of cell groups which are not included in his enumeration but which are at least as distinct as many of the "nuclei" which he has defined in the rabbit. It is clear that the personal factor is too obtrusive to allow of a numerical comparison in this way. Grünthal (8) has also laid some stress on the supposition that cyto-architecturally the hypothalamus appears more complex in the human foetus than in the adult, and he is disposed to draw an analogy between this phenomenon and his comparative anatomical results. Thus he figures a section through the hypothalamus of a 20-mm. foetus in which nine groups of cells are indicated, while in a larger embryo (80 mm.) of 4 months the cell density is noted to be much reduced. As he states, "der Umbau scheint also bereits im gang zu sein". It is suggested, in fact, that certain hypothalamic nuclei found in lower Mammals put in a transitory appearance during human ontogeny. This kind of evidence is clearly open to criticism. In early stages of development, the neurones all retain an embryonic character, and nuclear elements cannot be identified by criteria of cell type or of their relation to fibre paths (as yet mainly unformed). Homologies, therefore, can only be attempted by noting the relative positions of local condensations and comparing them with the topography of the nuclei in the adult brain. However, the very active ependymal proliferation at early stages gives rise to transitory clumpings of neuroblasts which need have no morphological value at all. Moreover, the outlines of these clumps of neuroblasts are sufficiently vague to demand the greatest caution in attempting to delineate them as separate "nuclei". My own studies of the brain in human embryos (ranging from an embryo of 28 mm. to a foetus of 5 months) have not confirmed Grünthal's conclusion that the hypothalamus in the embryo is more complex than it is in the adult.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Sagittal section of the hypothalamus close to the mid-line. Stained with toluidin blue. ×7.

Fig. 2. Sagittal section of the hypothalamus in the plane of the descending column of the fornix. Stained with toluidin blue. ×7½.

Plate II

Fig. 1. Sagittal section through the diencephalon of a 3½ months human foetus. Note the distinctness of the ventro-medial hypothalamic nucleus and the posterior hypothalamic nucleus, the latter extending upwards and backwards towards the motor tegmental area of the mid-brain. ×8½.

Fig. 2. Coronal section of the rostral part of the hypothalamus of a 5 months human foetus. Note the distinctness of the ventro-medial hypothalamic nucleus. ×4.

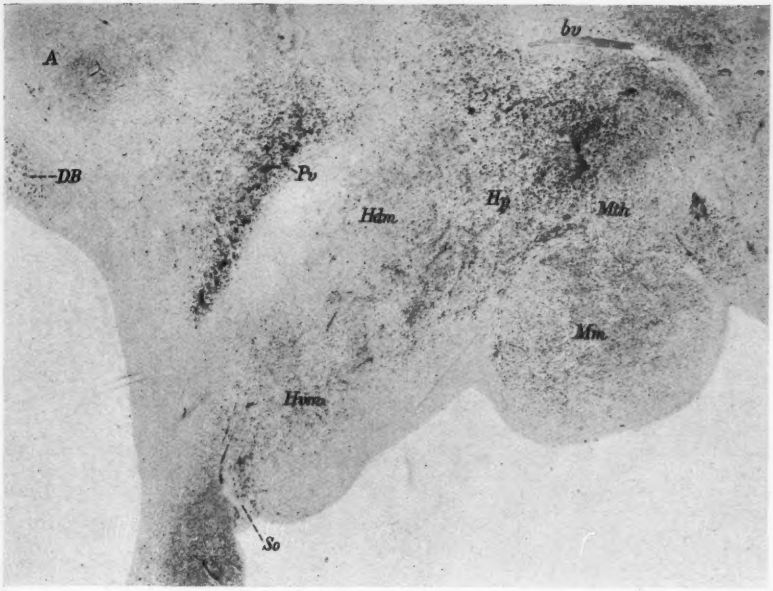
Fig. 3. Ventral aspect of the hypothalamus in an adult human brain showing the well-marked tubercles (indicated by arrows) produced by the nuclei tuberis.

Fig. 4. Cells of the paraventricular nucleus, stained with toluidin blue. ×100.

Fig. 5. Cells of the posterior hypothalamic nucleus, stained with toluidin blue, and showing the similarity of the large cells with those of the paraventricular nucleus. In this and the previous figure note the peripheral position of the Nissl granules and the frequent eccentric position of the nucleus. ×100.

LIST OF ABBREVIATIONS

A	Anterior commissure.	*Ml*	Nucleus mammillaris lateralis.
bv	Postero-median ganglionic artery.	*Mm*	Nucleus mammillaris medialis.
DB	Nucleus of diagonal band.	*Mth*	Mammillo-thalamic tract.
F	Fornix column.	*On*	Optic nerve.
Hdm	Nucleus hypothalamicus dorso-medialis.	*Pre*	Nucleus preopticus.
Hl	Lateral hypothalamic area.	*Pv*	Nucleus paraventricularis.
Hp	Nucleus hypothalamicus posterior.	*So*	Nucleus supraopticus.
Hvm	Nucleus hypothalamicus ventro-medialis.	*Tb*	Nucleus tuberis.
Int	Nucleus intercalatus.	*Th*	Thalamus.



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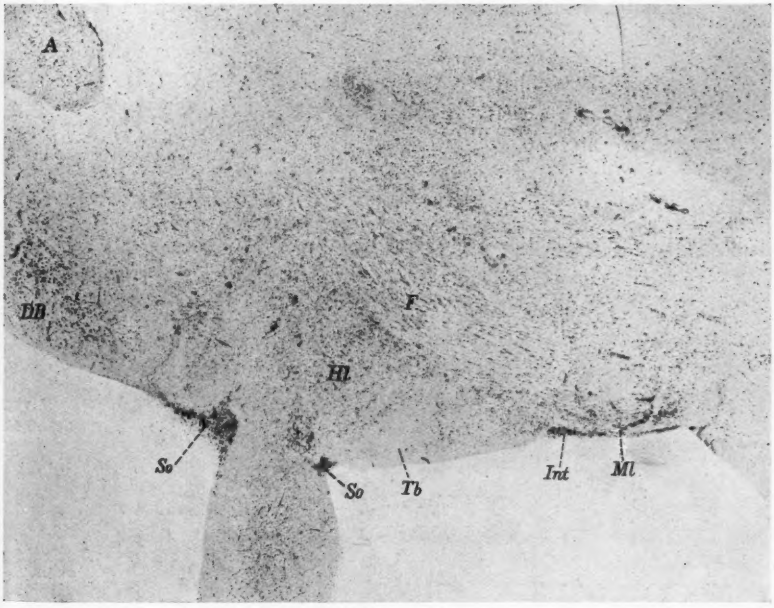

2

LE GROS CLARK—The Hypothalamic Nuclei in Man

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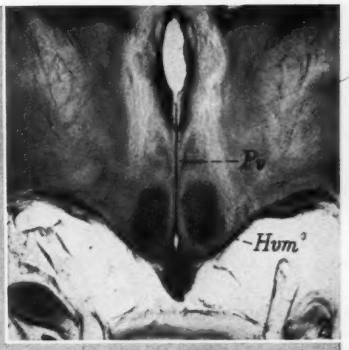

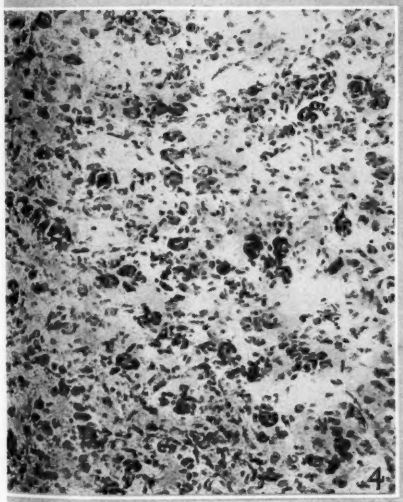

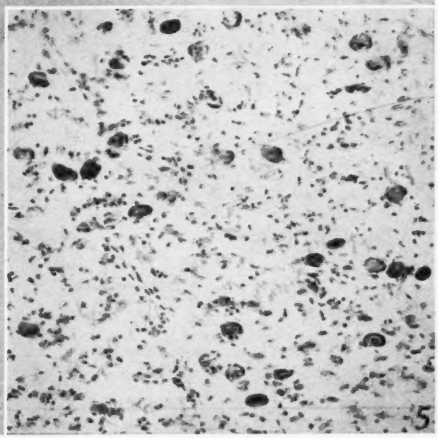

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OBSERVATIONS ON THE BLOOD SUPPLY AND THE INNERVATION OF THE AORTIC PARAGANGLION OF THE CAT

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In the course of studies on the distribution of the fibres of the aortic (depressor) nerve and the structure of its associated epithelioid body (Nonidez, 1935), I have paid much attention to the structure called paraganglion aorticum supracardiale by Penitschka (1931). In the cat it consists of irregular masses of roundish, epithelioid cells extending from the arch of the aorta to the base of the heart, in the space between the ascending aorta and the pulmonary artery. It was assumed that it received its blood supply from the aorta or from the coronary arteries. To my surprise, I found in a new-born kitten a distinct artery arising from the pulmonary trunk and sending branches to the cell masses of the aortic paraganglion. The study of the hearts of other kittens confirmed the observation.

On reviewing the limited literature on the subject I found that similar observations in kittens, in two human foetuses and a new-born infant have been reported recently by Palme (1934), who has succeeded in injecting the small artery to the paraganglion in adult cats. Since this remarkable blood supply may be of importance for understanding the function of the paraganglion, it seems advisable to present the anatomical evidence in some detail. A preliminary report on the innervation of this body will also be included in the present article, pending further investigations in other animals.

The material examined consisted of frontal sections of the hearts of four kittens, three prepared with the method of Cajal (fixation with chloral hydrate followed by alcohol ammonia), and one fixed with formol-bichromate and stained with haematoxylin. In the silver material the nerve fibres supplying the paraganglion and their terminations within the latter appear well impregnated, but owing to the thickness of the sections and to the low magnification employed they are indistinct in the photomicrographs (Plate I, figs. 1–4).

After fixation with formol-bichromate and staining with haematoxylin the paraganglion of the cat is seen to consist of at least two types of cells, namely, fairly large, spherical elements containing fine yellowish granulations in their cytoplasm, and smaller, more irregular cells without conspicuous granulations. The two cell types also occur in the carotid glomus and in the body I have termed the glomus aorticum, placed in the area of branching of the

aortic (depressor) nerve, above the arch of the aorta. The elements loaded with yellowish granules have been generally regarded as chromaffin cells, but this view is not shared by de Castro (1926), who has made microchemical tests in the carotid glomus and believes that the yellowish granulations are not chromaffin granules, but lipoids. On the other hand, in the paraganglion of the rabbit and guinea-pig no such cells exist, and the same holds true in a general way for the paraganglion of other forms studied by Penitschka. In silver impregnations the identification of the two cell types is not easy except for the difference in size, for the granulations disclosed by the fixation with formol-bichromate are not visible in these slides.

The paraganglion cells are arranged into roundish groups containing variable numbers of cells. Each group or lobule is surrounded by a thin connective tissue capsule which is not clearly separated from the adjacent interlobular stroma. The lobules are aggregated into larger masses or lobes of various sizes and shapes.

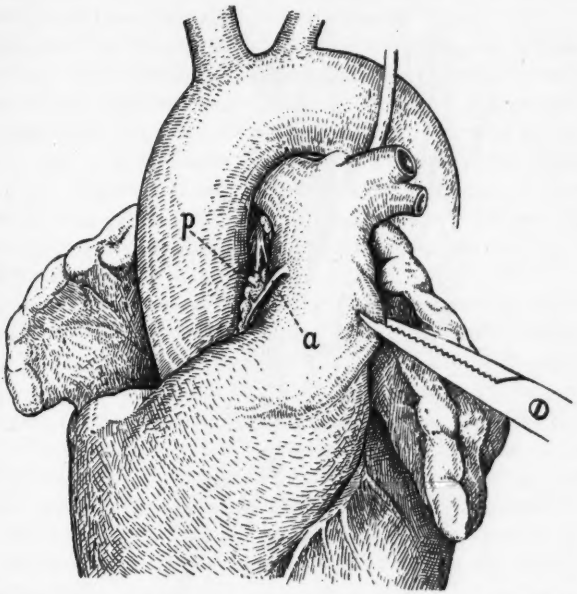
I. BLOOD SUPPLY

The position of the branch of the pulmonary artery supplying the aortic paraganglion is somewhat variable, as will be reported later. In favourable cases, when the small artery arises from the mesial aspect of the pulmonary trunk, it can be readily seen in injected hearts (text-fig. 1).

The origin of the branch to the aortic paraganglion in kitten No. 1 has been represented in text-fig. 2, reconstructed from camera lucida drawings. The proximal, extramural portion of the artery appears in Plate I, fig. 1, while its intramural portion is clearly seen in Plate I, fig. 2. In this particular case the artery under consideration arises from the mesial surface of the pulmonary trunk, approximately midway between the base of the trunk and its bifurcation into the two pulmonary arteries. The artery divides intramurally into two branches, *a* and a^1, respectively, the latter emerging from the pulmonary wall cephalad to the main branch, *a*. The position of this bifurcation within the wall of the pulmonary trunk has been indicated by broken lines in text-fig. 2. Branch a^1 is clearly seen in Plate I, figs. 1 and 2. The main branch *a* divides soon after emerging from the wall of the pulmonary trunk, sending a branch a^2 cephalad, and smaller branches ending in the masses of paraganglion cells (*g*) at this level. A second branch is seen coursing caudad, and bifurcating into two branches of unequal thickness, a^3 and a^4 respectively. The latter was traced to the large paraganglion masses located between the bases of the pulmonary artery and the aorta respectively, near the origin of the coronary arteries. These masses are not seen in the figures. A survey of the serial sections in this case has shown that small arterial branches issuing from the paraganglion artery enter the smaller masses or "lobules" and break into arterioles and capillaries. It is evident, then, that in this kitten the paraganglion received venous blood from the pulmonary trunk through the small artery described.

Equally convincing is the evidence on the origin from the pulmonary

artery of the paraganglion artery of kitten No. 2, shown in Plate I, figs. 3 and 4. The former figure shows the artery soon after it emerges from the wall of the pulmonary trunk, and a small branch is seen entering a paraganglion mass at the right of the artery. The intramural portion of the artery appears in Plate I, fig. 4. In this particular case the paraganglion artery arises from the pulmonary trunk near the base of the ductus arteriosus, a portion of which appears in the figure (*d*). Such being the case most of the branches of the artery run caudad, that is, toward the base of the heart. A fairly large branch, however, was



Text-fig. 1. Arterial branch (*a*) from the pulmonary trunk to the aortic paraganglion (*p*). The latter appears embedded in adipose tissue, hence its apparently large size. Adult cat. From an injected preparation by Dr Walter Modell.

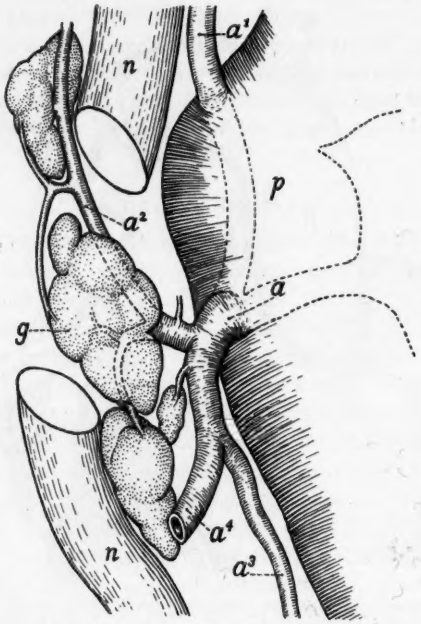
noticed running almost horizontally, and after reaching the posterior (dorsal) aspect of the aorta was seen to supply a number of retro-aortic paraganglion masses.

In kitten No. 3 the artery was also traced to the distal portion of the pulmonary trunk, near the point of its division into right and left pulmonary arteries, but unfortunately no sections showing its intramural course had been mounted. The evidence in this case is, therefore, incomplete.

Kitten No. 4 is of interest because it shows a different condition, which may account for the failure to find a distinct artery issuing from the pulmonary trunk in some cats. In this case the paraganglion is supplied by two distinct branches arising from the proximal portion of the right pulmonary artery.

Of these branches one springs from the upper (cephalic) surface of the artery and descends nearly parallel to the pulmonary and the ascending aorta. The second, smaller branch, leaves the lower (caudal) surface of the pulmonary artery and supplies a few retro-pulmonary groups of paraganglion cells. A similar case in which the paraganglion artery sprang from the right pulmonary has been reported by Palme.

Veins are abundant among the paraganglion masses, and run more or less parallel to the pulmonary trunk. Palme believes that most of the veins from



Text-fig. 2. Reconstruction of the proximal portion of the paraganglion artery of a new-born kitten (No. 1). Intramural course of artery indicated by broken lines. *a*, main arterial branch; a^1, a^2, branches coursing cephalad to end in paraganglion masses below the arch of the aorta; a^3, a^4, branches running caudad; *g*, paraganglion masses; *n*, cardiac nerve, cut to disclose underlying structures; *p*, pulmonary trunk. Reduced. (See also Plate I, figs. 1 and 2.)

the paraganglion empty in the right atrium and ventricle. In kitten No. 1 the larger vein seen in Plate I, figs. 1 and 2, *v*, had valves (not seen in the figures) directed cephalad, a fact suggesting that it may be a tributary of the superior vena cava. Further observations on injected hearts will be necessary to learn more about the venous drainage of the paraganglion. The same may be said in regard to the possibility of a systemic blood supply to the body just mentioned. My observations indicate the existence of small arteries reaching the paraganglion with the bundles of nerve fibres, but I have not been able to

trace definitely their origin. Another possible source of arterial blood is the vasa vasorum of the pulmonary trunk and the ascending aorta.

II. INNERVATION

The nerve supply to the human aortic paraganglion has been recently described by Seto (1935). Since my own findings on this subject are at variance with those reported by the author named, they will be briefly considered in the present article. This divergence of opinion may be due not only to the different material but also to the technique, since Seto used the method of Bielschowsky and I have employed the method of Cajal.

The innervation of the aortic paraganglion is best studied in frontal sections of the heart and large vessels of new-born animals, in which the nerve bundles can be traced for relatively long distances. The main source of nerve fibres for the paraganglion is a stout nerve trunk, the right cardiac nerve (text-fig. 2 and Plate I, figs. 1–4, *n*), which descends between the aorta and pulmonary to the base of the heart. Here it breaks into large branches, many of which are connected with ganglia of various sizes, located for the most part in the connective tissue between the bases of the large vessels and the vicinity of the coronary sinus.

The cardiac nerve carries very numerous, thin, unmyelinated fibres, derived in great numbers from the sympathetic (inferior cervical, stellate ganglion). In material prepared with the method of Cajal (fixation with chloral hydrate followed by alcohol ammonia), these fibres stain light brown, whereas the thicker, mostly myelinated fibres, appear dark brown to black. The latter are quite numerous; they seem to be derived from the vagus at the level of emergence of the recurrent nerve. From the mixed cardiac nerve (vagus and sympathetic) arise bundles of fibres which course toward the masses of epithelioid cells constituting the paraganglion. The bundles mentioned vary considerably in direction and diameter, and may follow the branches of the paraganglion artery.

Not all the fibres in the bundles under consideration are destined to the innervation of the aortic paraganglion, for many of them, mostly thin and medium-sized, are preganglionics ending around neurones occurring in the bundles, outside or within the paraganglion. These are the ganglion cells seen by Penitschka and also by Palme. They are of variable size and shape, though clearly multipolar, are enclosed within capsules and form groups of diverse size. With the routine methods of staining they may appear scattered among the paraganglion cells. The silver technique, however, discloses that such is not the case, for wherever they occur they are associated with nerve bundles, some of which may enter the paraganglion. Without recourse to the experimental (degeneration) methods it is impossible to say whether the preganglionics are derived from the vagus or from the sympathetic. The destination of the axons of the ganglion cells is also a problem which cannot be settled by mere histological study.

As the bundles of medium-sized and thick nerve fibres reach the masses of paraganglion cells they separate to form a rich superficial plexus from which thin bundles enter among the lobules or smaller groups of paraganglion cells. The thinner or secondary bundles ultimately break up, and independent fibres course in every direction on the surface of the lobules and penetrate among their cells (Plate II, fig. 1). Since the branching of the individual fibres takes place for the most part in the periphery of each lobule, and since the branches penetrate among crowded cells, they may give rise to nests or baskets surrounding one or more cells (Plate II, figs. 2 and 3).

Reticulated swellings of various sizes and shapes are of common occurrence in branches of the nerve fibres, especially near the periphery of the paraganglion lobules. They may occur in either the thin or the thicker branches of an arborisation. An example of swellings in the thin branches has been represented in Plate II, fig. 4. In some instances they appear as round or club-shaped thickenings, some of which are nearly as large as the paraganglion cells and, on superficial examination, might be mistaken for neurones (Plate II, fig. 5). Upon careful focusing a nucleus may be seen immediately above or below the swelling. A study of a considerable number of these enlargements has shown that they are, in most cases, cup-shaped swellings and that they may be closely applied against the surface of the epithelioid elements composing the paraganglion. When seen on edge they have the appearance depicted in Plate II, fig. 6. The meaning of these voluminous enlargements is not clear, but since they were found in new-born kittens they may represent material to be used for further growth of the terminal arborisations of the nerve fibres. Similar swellings occur also in the epithelioid bodies located above the aorta (aortic glomi), which will be described in another article. In other cases the arborisations lack swellings (Plate II, fig. 8).

According to Seto the interstices between the paraganglion cells are occupied by a terminal reticulum "das sich dicht an der Oberfläche der Parenchymzellen ausbreitet". With the method of Cajal no terminal reticulum is visible, except those enlarged, finely reticulated portions of the branches already mentioned. Instead of the formation reported by Seto, this procedure discloses the presence of very thin, usually wavy twigs coursing in every direction among the paraganglion cells and ending as minute rings or small, reticulated clubs in contact with the surface of the cells. The rings are clearly visible in good impregnations, and their relation to the finer twigs could be confirmed in dozens of cases. They vary in size, though not considerably. A few of these terminal structures have been copied in Plate II, fig. 9. These rings and clubs are identical with the terminations of similar type described by de Castro (1926) in the glomus caroticum. That they are not artifacts produced by the Cajal technique is shown by the fact that they have been demonstrated with the Bielschowsky technique in the carotid glomus by Riegele (1928) and Boeke (1932).

Since thin, unmyelinated fibres course in the nerve bundles supplying the

paraganglion, the question naturally arises whether the minute rings mentioned above are the terminations of unmyelinated fibres, or whether they are also the endings of large and medium-sized fibres. This point is of importance for the problem of the possible participation of the sympathetic in the innervation of the paraganglion. Accordingly, I have studied carefully a good number of arborisations of thick fibres, tracing their finer branches to their termination whenever it was possible. Among the clearest cases is the branching of the thick myelinated fibre copied in Plate II, fig. 7. It occurred in the periphery of a small bundle entering one of the paraganglion lobules. From one of the thick branches arises a thin twig ending as a delicate ring (*e*) in contact with the surface of a cell. Similar cases could be illustrated if space permitted. It follows, then, that the thick fibres participate along with the medium-sized and thin in the formation of the finer twigs coursing among the cells of the paraganglion and ending as rings or small club-shaped enlargements. In fact, surprisingly thin twigs are seen sometimes arising directly from thick branches.

The presence of sensory arborisations in the arterial branches supplying the paraganglion has also been reported by Seto in the human. Our observations with two different methods of silver impregnation agree in this respect, for nerve endings of the pressor-receptor type undoubtedly occur in the paraganglion artery of the cat. They closely resemble the pressor-receptors found in the area of distribution of the aortic (depressor) nerve, and may perform a similar function.

III. DISCUSSION

The unusual type of blood supply to the aortic paraganglion indicates that this body must perform activities intimately connected with the blood flowing through the pulmonary artery. Palme, however, seems to think that the pulmonary origin of the paraganglion artery is not constant, but that it constitutes a common variety ("Er stellte sich heraus, dass es sich um eine rechte häufige Varietät handelt", p. 404). This implies that in other instances the paraganglion may receive its blood from other sources, such as the aorta and the coronary arteries.

In the human material examined by Palme the paraganglion artery was seen to arise in three cases from the base of the pulmonary trunk above the area of the valves, but these observations could not be confirmed in other hearts. However, since in the latter no conspicuous blood supply from other sources is reported, there is reason to believe that the paraganglion *might* have received its blood from the pulmonary trunk through arteries hidden from view by the surrounding tissues or too small to be readily detected. In this connection kitten No. 4 of the present article is of interest, for in this instance there were two small arterial branches arising from the right pulmonary artery instead of the pulmonary trunk. The possibility of wide variations in this regard must be taken into account. Furthermore, in the adult the method of dissection, even when following injection of the vessels, may not yield conclusive results, since the paraganglion artery is really very small.

As far as three of the kittens considered in this article are concerned, the evidence on the pulmonary origin of the paraganglion artery is conclusive. If, as suggested by its constant presence in all the Mammals studied so far, the aortic paraganglion performs a definite function of importance for the organism, it seems hardly possible that it can discharge its activities regardless of the source of its blood supply. This is particularly true if, as claimed by Seto, the body under consideration is a gland producing a secretion which causes a fall in the blood pressure. That the paraganglion may, in addition, receive arterial blood from the aorta or its branches is not questioned; indeed, there are indications pointing in this direction, but if it can be proved that it always receives a branch from the pulmonary artery then its abundant venous blood supply must be of fundamental importance in connection with its physiological role, as is the case in the liver.

On the other hand, the innervation of the aortic paraganglion is very similar to the nerve supply to the carotid glomus. The presence of numerous arborisations of medium-sized and thick nerve fibres among the epithelioid cells strongly suggests that the nerve bundles supplying this body are not primarily derived from the sympathetic, but that large numbers of their fibres are conveyed by the vagus. Whether these fibres are afferent, as is the case in the carotid glomus (de Castro, 1928), requires experimental demonstration. I may point out, however, that the mode of termination of the nerve fibres in the aortic paraganglion is identical with that described by de Castro (1926) for the carotid glomus, in which baskets of fibres around the epithelioid cells and reticulated swellings also occur, especially in the cat (de Castro, 1926; Watzka, 1934).

My observations in regard to the terminations of the nerve fibres within the paraganglion are in sharp contrast with the conclusions of Seto. No terminal reticulum can be demonstrated with the method of Cajal, except the reticulated swellings occurring in the course of the nerve twigs (Plate II, figs. 2–4). Seto's fig. 6 is unconvincing, inasmuch as it is impossible to identify the branches of the nerve fibres. After a careful study of this figure one gains the impression that the so-called terminal reticulum may also contain large numbers of fine argyrophile connective tissue fibres, which as is well known, stain with the method of Bielschowsky and cannot always be decolorised completely with diluted acetic acid. Similar images are obtained with other methods of silver impregnation which do not stain the nerve fibres (Achúcarro's modified Bielschowsky; Rio Hortega's silver carbonate method).

The presence of afferent nerve endings of pressor-receptor type in the small artery supplying the aortic paraganglion is significant, for similar terminations are known to occur in the arteries to the carotid glomus. Furthermore, since the pulmonary trunk lacks a definite pressor-receptor area of its own (Nonidez, 1935), it is quite possible that the terminations in the paraganglion artery are destined to receive stimuli caused by changes in the blood pressure. This would explain the existence of vascular reflexes identical with those mediated by the

aortic (depressor) nerve—elicited by increase in the blood pressure within the pulmonary artery—as has been demonstrated by the physiologists.

Since there are no essential differences in the structure and the innervation of the aortic paraganglion and the carotid glomus, the identity of these two bodies, suggested by Penitschka, can no longer be questioned. In the light of recent experiments on the function of the carotid glomus, considered in my previous article, it would appear that the aortic paraganglion may be a chemoreceptor for the pulmonary artery. The nerve endings on its cells would be stimulated by changes in the CO_2 content of the blood which it receives from the pulmonary artery through the small branch described in the present article. If such be the case, the term glomus pulmonalis seems more appropriate since the relation of this body to the aorta is simply one of proximity.

IV. SUMMARY

1. A small arterial branch to the aortic paraganglion of the cat, arising directly from the pulmonary trunk or one of the pulmonary arteries, is described (text-figs. 1, 2 and Plate I, figs. 1–4). This branch, recently reported by Palme (1934) in the cat and human, supplies the paraganglion with venous blood.

2. A preliminary study of the innervation of the aortic paraganglion indicates that this body is supplied by numerous thick and medium-sized nerve fibres, probably derived from the vagus. The fibres branch repeatedly on the surface of the groups of paraganglion cells (Plate II, fig. 1) and have reticulated swellings (Plate II, figs. 2–6). The finer twigs end in contact with the epithelioid elements by means of minute rings or small, reticulated clubs (Plate II, figs. 2, 3, 7 and 9). No terminal reticulum, reported by Seto (1935) in the human, occurs in the cat.

3. Since there are no essential differences in the structure and innervation of the carotid glomus and the aortic paraganglion, it is suggested that the latter may be a chemoreceptor for the pulmonary artery. If such be the case the term glomus pulmonalis is proposed as more appropriate.

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EXPLANATION OF PLATES

Plate I

Figs. 1 and 2. Photomicrographs showing branch (*a*) from the pulmonary artery (*p*) to the paraganglion aorticum (*g*). a^1–a^3, branches of paraganglion artery; *ao*, ascending aorta; *n*, cardiac nerve; *v*, vein. (Kitten No. 1, frontal sections; Cajal method.) (See also text-fig. 2.)

Figs. 3 and 4. Photomicrographs showing branch (*a*) from the pulmonary artery (*p*) to the aortic paraganglion (*g*). *d*, ductus arteriosus; *n'*, nerve bundles to paraganglion. Other letters as in preceding figure. (Kitten No. 2, frontal sections; Cajal method.)

Plate II

All figures are drawings made under a Zeiss hom. imm. obj. 60 (n.a. 1·4) and compensating oculars 7 (fig. 1), 15 (figs. 5–8) and 20 (figs. 2, 3, 4 and 9). Several planes have been represented. Reduced for reproduction. Cajal method. No nuclear stain used.

Fig. 1. Bundles of nerve fibres and plexus in a position of the aortic paraganglion adjacent to an arterial branch (*a*) labelled a^4 in text-fig. 2.

Figs. 2 and 3. Pericellular baskets formed by branches of nerve fibres around paraganglion cells.

Fig. 4. Reticulated thickenings in a branch of a nerve fibre.

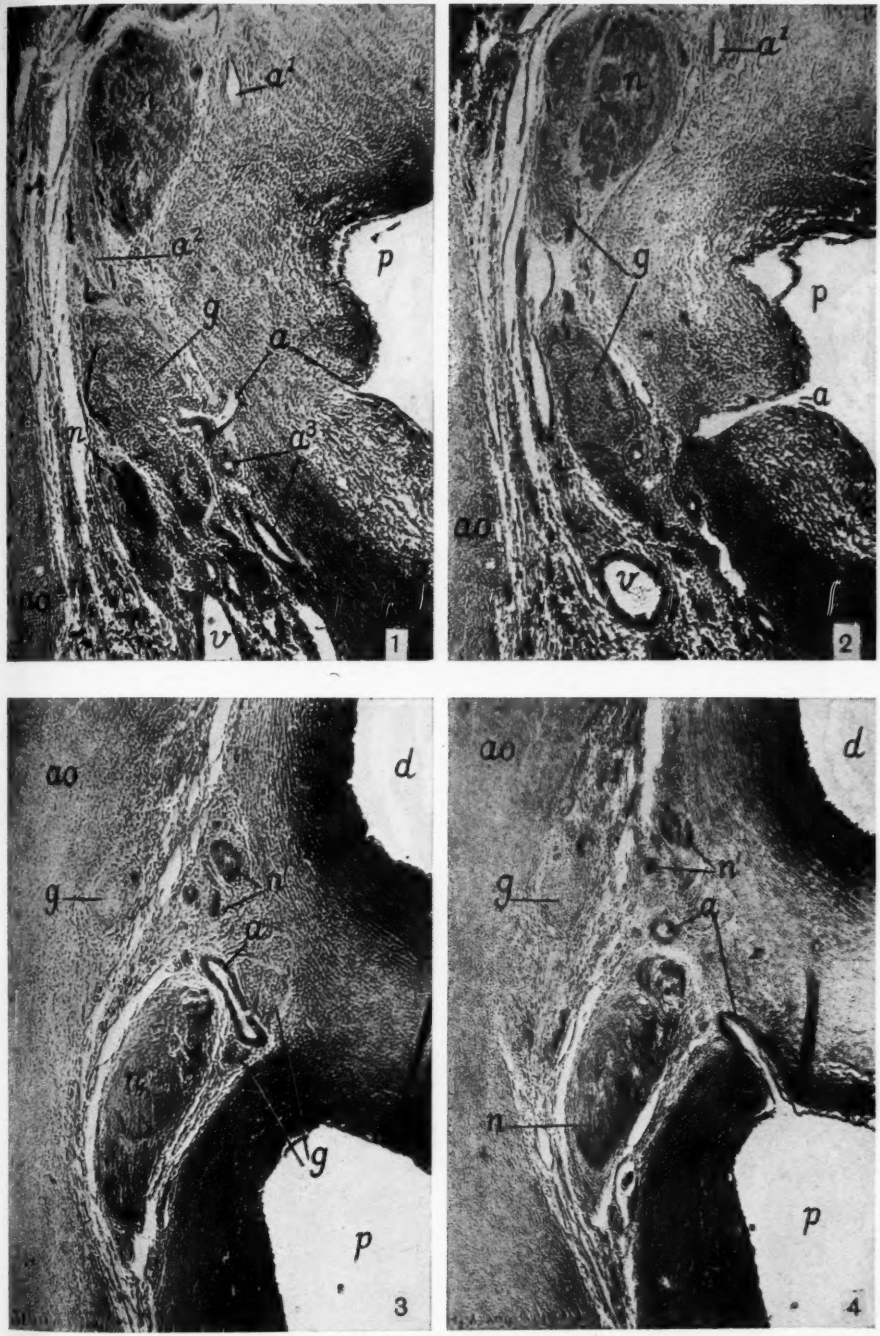
Fig. 5. Large reticulated enlargements in nerve fibres.

Fig. 6. Side view of large reticulated swellings closely applied against the paraganglion cells.

Fig. 7. Thick myelinated fibre branching on the surface of a paraganglion lobule, showing termination (*e*) of a twig.

Fig. 8. Collateral of a medium-sized nerve fibre branching on the surface of a paraganglion lobule.

Fig. 9. Terminal rings and clubs in twigs of nerve fibres.



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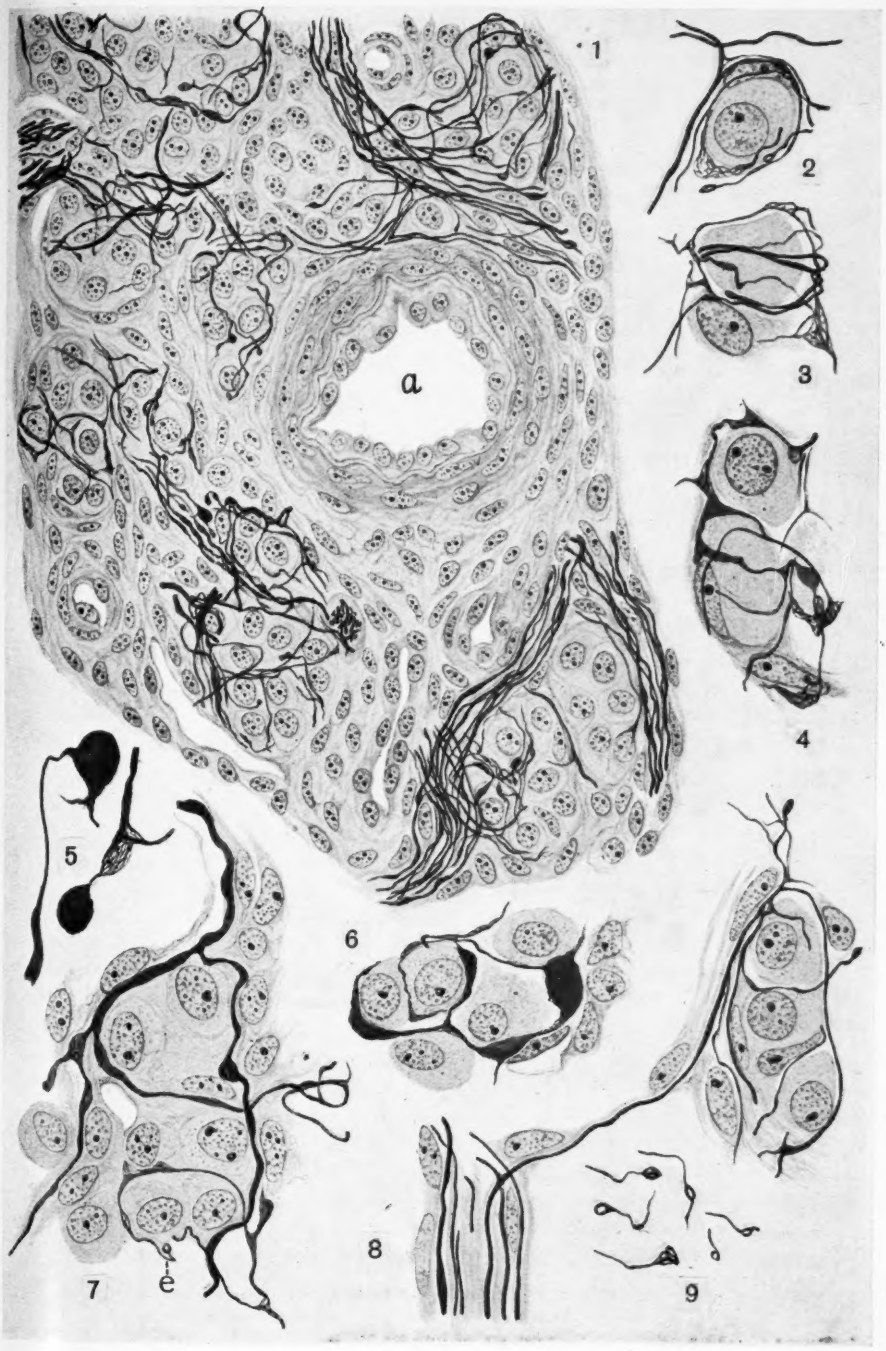

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SYMPATHETIC AND PARASYMPATHETIC NERVES IN THE ORBIT OF THE CAT

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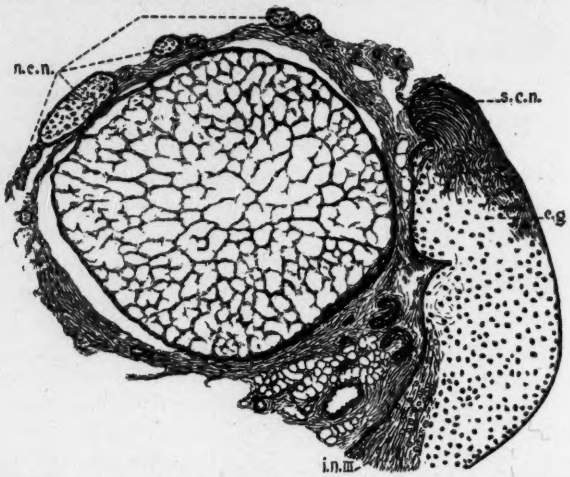
INTRODUCTION

Cranial autonomic ganglia in mammalian species commonly are described as having three roots, viz. a motor, a sensory and a sympathetic root. The motor root carries pre-ganglionic fibres which arise in the brain stem and terminate in the ganglion in synaptic relationship with ganglion cells. The sympathetic root consists of the post-ganglionic sympathetic fibres which arise in the superior cervical ganglion and extend cephalad *via* the internal carotid plexus. The sensory root consists of afferent cranial nerve components. Both the sympathetic and the sensory fibres pass through the ganglion without interruption and become incorporated in the nerves which arise from it. The motor and sympathetic roots of the spheno-palatine ganglion, for example, are derived from the nerve of the pterygoid canal. This nerve is formed by the union of the great superficial petrosal nerve, which conveys pre-ganglionic fibres, and the deep petrosal nerve, which conveys sympathetic fibres. The sensory root is composed of the spheno-palatine nerves, which convey most of the sensory fibres which pass through the spheno-palatine ganglion. In view of these anatomical relationships, it would be impossible, by surgical intervention, to eliminate the parasympathetic fibres to the organs innervated by the nerves from the spheno-palatine ganglion and still retain the sympathetic supply to these organs, because removal of the spheno-palatine ganglion or section of the nerves arising from it would interrupt not only the parasympathetic fibres which arise in the ganglion but also sympathetic fibres which pass through it.

The anatomical relationships of the ciliary ganglion, according to most accounts, are comparable to those of the spheno-palatine. The inferior ramus of the oculomotor nerve is designated as the short or motor root; fibres derived from the cavernous plexus compose the sympathetic root; and fibres derived from the nasociliary nerve constitute the long or sensory root. The sympathetic root usually fuses with the sensory root before entering the ganglion; consequently, branches of the nasociliary nerve contain both sensory and sympathetic fibres. Removal of the ciliary ganglion in such cases results in degeneration not only of the sympathetic fibres but also of all sympathetic fibres passing through the ganglion. It is obvious that whenever most of the

sympathetic fibres reach the eye by way of the ciliary ganglion,[1] the parasympathetic fibres cannot be destroyed without complete destruction of the sympathetic fibres associated with them. In certain species the relationships of the nerves to the ciliary ganglion undoubtedly are as summarised above. A study of the ciliary ganglion in the cat, however, shows that in this species the relationships indicated above do not obtain except with respect to the motor root. In this species sympathetic fibres usually do not traverse the ciliary ganglion. Jegerow (1887) failed to detect a root to the ciliary ganglion in cats. Anderson (1905) also failed to find one. Dupas (1924) states that the



Text-figs. 1–3. A series of sketches of sections of orbital nerves, made by bleaching retouched photomicrographs. These sections were selected from the serial sections of one orbit.

Text-fig. 1. The ciliary ganglion is shown with fibres of the third nerve entering and the fibres of the short ciliary nerves leaving the ganglion. Branches of the nasociliary nerve are found above and to one side of the optic nerve.

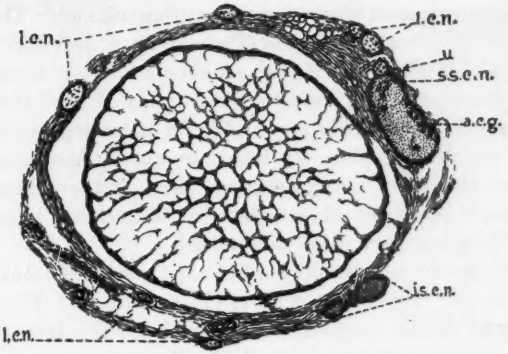
ciliary ganglion receives directly only a single root which is the motor one. He found macroscopically no sympathetic root, although he thought that sympathetic fibres might reach the ganglion through the motor root.

OBSERVATIONS

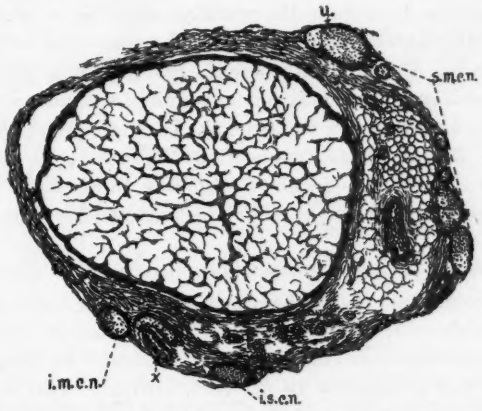
Sections of the ciliary ganglion. Serial sections of the ciliary ganglion of the cat prepared by the pyridine silver method reveal no bundles of sympathetic or sensory nerve fibres passing through the ganglion. Since the neurons are widely separated from one another in the ciliary ganglion (Plate I, fig. 1), the types of fibres and their distribution within the ganglion can be readily studied. No fibres of very large or very small diameter are present and except

[1] In serial sections of the one orbit of a monkey practically all of the sympathetic fibres to the eye did join the ciliary ganglion.

for terminal pericellular networks about the neurones, within the ganglion, all fibres appear to be myelinated. In none of the ganglia of the cat studied could bundles of small, unmyelinated fibres be found within the ganglion and no nerves with unmyelinated fibres joined any part of the ciliary ganglia studied in serial sections. In a series of sections of the ciliary ganglion of the monkey (Plate I, fig. 2), by contrast, one or more small nerves each containing



Text-fig. 2. Long ciliary nerves joining the supero-lateral short ciliary nerve and its rami.



Text-fig. 3. Long ciliary nerves joining the inferior short ciliary nerve and its rami.

unmyelinated fibres, could be traced to the ganglion. As these bundles are traced through the ganglion, they remain intact until they enter the short ciliary nerves.

Sections of the oculomotor and ciliary nerves. A study of serial sections of the nerves connected with the ciliary ganglion of the cat likewise shows a dissociation of parasympathetic nerve fibres from the sympathetic and sensory nerve fibres, at least close to the ganglion.

The ramus of the oculomotor nerve (Plate II, fig. 4) which carries the pre-ganglionic fibres to the ciliary ganglion consists chiefly of large and small myelinated fibres. Pyridine silver preparations of this ramus (Plate II, fig. 3) reveal few unmyelinated fibres or none at all. The smaller myelinated fibres, which become aggregated at one side of the nerve, are those which enter the ganglion as pre-ganglionic fibres from the oculomotor nerve.

The sections of the short ciliary nerves taken near the ganglion are composed entirely of small myelinated fibres of nearly uniform diameter. This observation has been confirmed in both osmic acid (Plate II, fig. 6) and silver preparations (Plate II, fig. 5). Myelination of post-ganglionic fibres is uncommon except in the short ciliary nerves. The histological appearance of the short ciliary nerve, therefore, is particularly favourable for detecting sympathetic fibres wherever any are present, since the latter are mainly unmyelinated.

Sections of the short ciliary nerves taken farther distally, however (text-figs. 2 and 3), in almost all cats, show that branches of long ciliary nerves unite with them before they reach the eyeball.

The long ciliary nerves are composed of large myelinated, small myelinated (Plate II, fig. 8) and unmyelinated fibres (Plate II, fig. 7). The unmyelinated fibres probably are in part sympathetic, and the larger myelinated ones sensory.

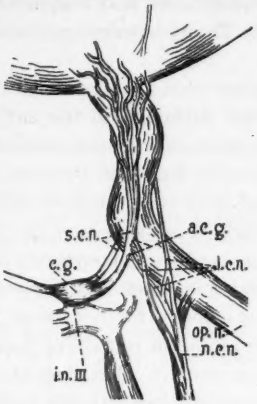
Whenever a union of long and short ciliary nerves occurs, the large myelinated fibres (text-fig. 1) and the unmyelinated fibres from the nasociliary nerves can always be detected in the resulting mixed nerve, since they appear in sharp contrast to the smaller, myelinated parasympathetic fibres of the short ciliary nerves. At the places where the first long ciliary nerves superiorly join the short ciliary nerves, it is not uncommon to find scattered ganglion cells in the short ciliary nerves (text-fig. 2). These are designated as accessory ciliary ganglia. The functional connections of these cells undoubtedly are affected through pre-ganglionic fibres from the third nerve which traverse the ciliary ganglion and the short ciliary nerves until the cells are reached.

The ciliary ganglion and the ciliary nerves—gross dissection

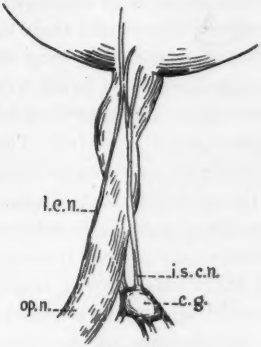
The microscopic findings as described above were confirmed in a series of dissections made with the aid of a dissecting microscope (text-figs. 4–7).

The demonstration of the ciliary nerves is facilitated by treating the orbital contents, fixed in a solution of 10 per cent. formalin, washed in water, and then partially dissected with a 0·5 per cent. solution of silver nitrate for about 12 hours. In such material the nerves appear dark brown while other tissues have a very light brownish colour. The darkness of the other tissues is greater when stronger silver solutions are used; when the treatment with the silver nitrate is prolonged; or when the silvered material has been exposed to the light for long periods of time. If dissections are made under water with the use of a dissecting microscope and a projection lamp the differentiation, however, remains good for a long time providing the material, when not in use, is kept in distilled water in a dark place. The ganglia in the preparations after

being in the silver nitrate are usually much lighter than the nerves; consequently with the use of the microscope even the position of accessory ciliary ganglia of any size can be readily determined.



Text-fig. 4.



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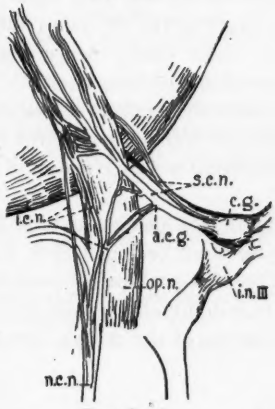

Text-fig. 6.

Text-fig. 7.

Text-figs. 4–7. A series of sketches of dissections of orbital nerves made from photographs. The relations of ciliary ganglion, short ciliary nerves, and long ciliary nerves are shown.

The main ciliary ganglion is a small, flattened, ovoid or triangular body located in the deep part of the orbit, lateral to the optic nerve and superior to the inferior ramus of the oculomotor nerve. From the inferior ramus of the oculomotor nerve, pre-ganglionic fibres enter the ganglion where they form synapses with ganglion cells. Besides the inferior ramus, no other nerves joining the ganglion have been found. The nerves which arise from the ganglion usually consist of two short ciliary nerves which extend forwards from the anterior border of the ganglion. The superior one, which is the larger, lies on the lateral

surface of the optic nerve in the first part of its course and then on the lateral and supero-lateral surface, where it divides into numerous small branches. The inferior and smaller one usually bifurcates as it leaves the ciliary ganglion. The two branches then pass lateral to the optic nerve and beneath it where they also divide into a number of small nerves. These, however, are never quite as numerous as those of the superior group.

The ciliary nerves and their branches course along the optic nerve to the eyeball together with the long ciliary nerves. Although in the cat no long ciliary nerves have been found to join the ganglion, it is not uncommon to find both short ciliary nerves or their branches joined by long ciliary nerves between the ganglion and the eyeball. The number of long ciliary nerves is variable. Two branches or a bifurcated branch or a single nerve may join the proximal third of the superior short ciliary nerve, and it is at these points of union that accessory ciliary ganglia usually occur. Beyond this region superiorly a variety of conditions may appear from a complete absence of further union between long and short ciliary nerve branches to a union of all remaining long ciliary branches with short ciliary branches. The latter usually takes place close to the eyeball. In the case of the ciliary nerve branches beneath the optic nerve where they are not so numerous, only single or double long ciliary nerves appear, and they may not join the short ciliary nerve branches until close to the eyeball or they may form with the short ciliary nerve branches a simple network of fibres along the distal two-thirds of their length.

Whenever union of long ciliary branches with short ciliary branches occurs, mixed nerves result which contain sympathetic and sensory fibres from the former and parasympathetic fibres from the latter (a section is shown in Plate II, fig. 9).

In the preceding description a summary of the general details only has been attempted. Specific individual details have not been included. It is to be pointed out, however, that variations in the relationships between long and short ciliary nerves differ from individual to individual, and also between the nerves of the orbit of the one side and the nerves of the orbit of the opposite side of the same individual.

SUMMARY

The results of the anatomical study may be summarised as follows:

1. The so-called sympathetic and the sensory roots of the ciliary ganglion are not found in the cat.

2. The short ciliary nerves when they first arise from the ciliary ganglion contain only post-ganglionic parasympathetic fibres.

3. Sympathetic fibres become incorporated in the short ciliary nerves only after those nerves have been joined by long ciliary nerves somewhere between the ganglion and the eyeball.

4. In the orbit of the cat there is at least a partial separation of post-ganglionic sympathetic and parasympathetic fibres which innervate the eye:

(*a*) since the ciliary ganglion has no sympathetic fibres passing through it; (*b*) since short ciliary nerves are separate for some distance.

It follows from these conclusions that, by careful operative procedure, the eye can be deprived of its post-ganglionic parasympathetic supply by removal of the ciliary ganglion without great disturbance to its sympathetic supply.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Photomicrograph of a typical section through the ciliary ganglion of a cat.

Fig. 2. Photomicrograph of a section through the ciliary ganglion of a monkey which shows a long ciliary nerve in section that passes through the ganglion.

Plate II

Fig. 3. Photomicrograph of a section of the inferior ramus of the oculomotor nerve. Pyridine silver method.

Fig. 4. Photomicrograph of a section of the inferior ramus of the oculomotor nerve. Osmic acid method.

Fig. 5. Photomicrograph of a section of one of the short ciliary nerves, taken near the ciliary ganglion. This nerve was photographed at a magnification higher than that used for other nerves on this plate. Pyridine silver method.

Fig. 6. Photomicrograph of a section of one of the short ciliary nerves taken near the ganglion. Osmic acid method.

Fig. 7. Photomicrograph of a section of a long ciliary nerve. Pyridine silver method.

Fig. 8. Photomicrograph of a section of a long ciliary nerve. Osmic acid method.

Fig. 9. Photomicrograph of a section of a mixed ciliary nerve. A short ciliary nerve has been joined by long ciliary nerves, whose large myelinated sheaths show clearly.

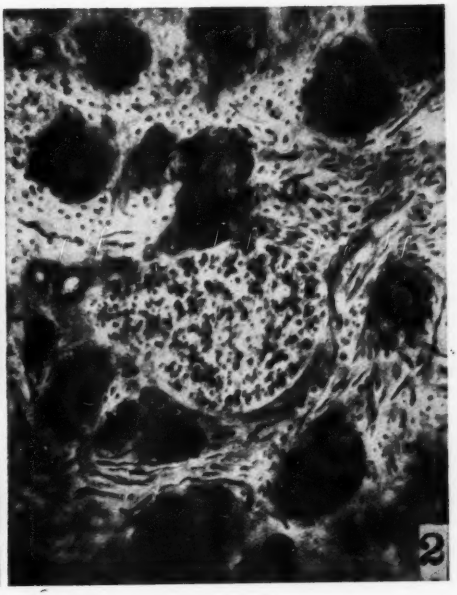
ABBREVIATIONS

Text-figs. 1–3

a.c.g. Accessory ciliary ganglion.
c.g. Ciliary ganglion.
i.m.c.n. Inferior mixed ciliary nerves.
i.n.III. Fibres of inferior ramus, oculomotor nerve entering the ciliary ganglion.
i.s.c.n. Inferior short ciliary nerves.
l.c.n. Long ciliary nerves.
n.c.n. Branches of nasociliary nerve.
s.c.n. Fibres of short ciliary nerves leaving the ciliary ganglion.
s.m.c.n. Supero-lateral mixed ciliary nerves.
s.s.c.n. Supero-lateral short ciliary nerves.
u. Points of union between supero-lateral short ciliary nerves and long ciliary nerves.
x. Points of union between inferior short ciliary nerves and long ciliary nerves.

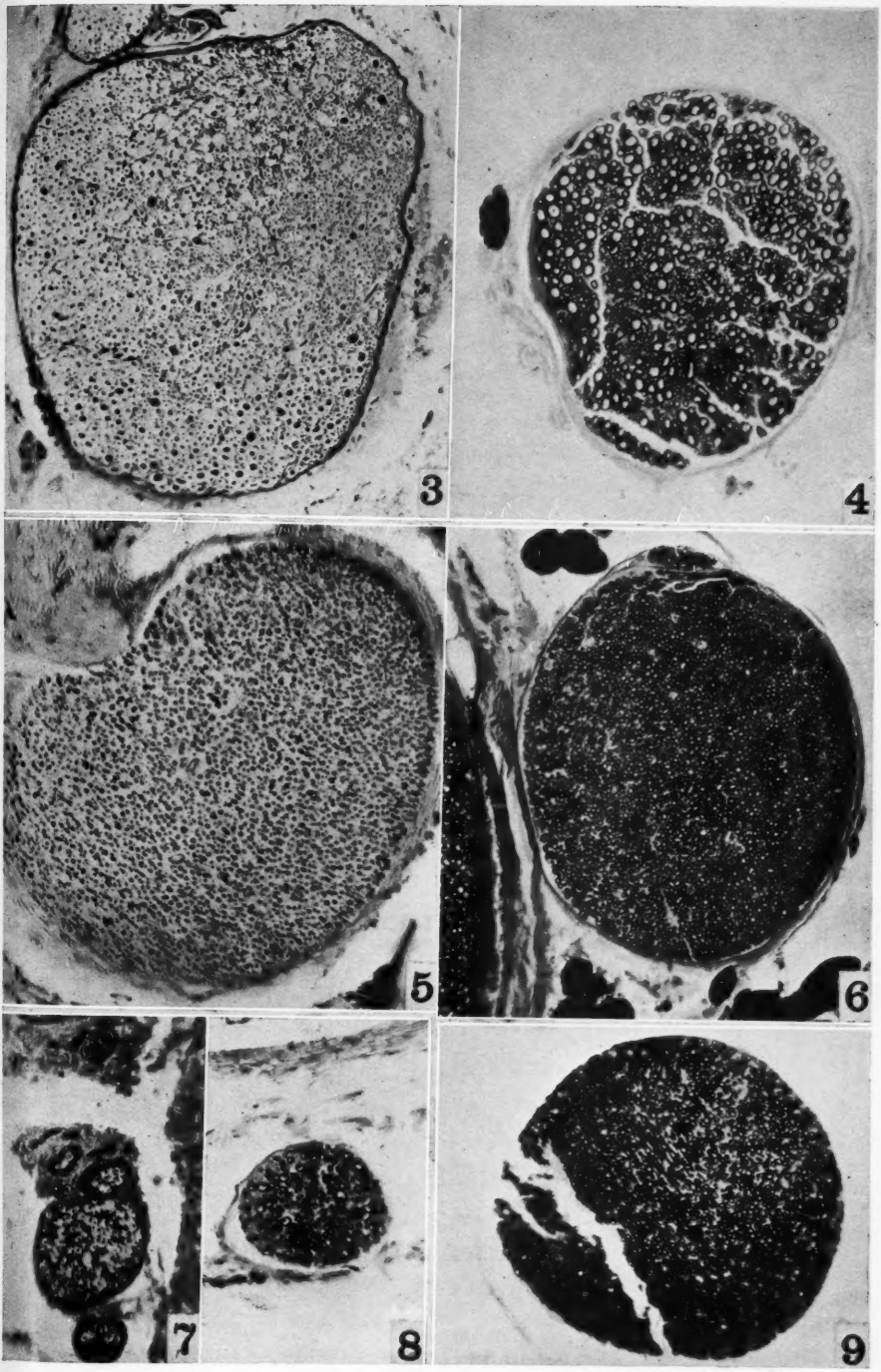
Text-figs. 4–7

a.c.g. Accessory ciliary ganglion.
c.g. Ciliary ganglion.
i.n.III. Inferior ramus, oculomotor nerve to ciliary ganglion.
i.s.c.n. Inferior short ciliary nerves.
l.c.n. Long ciliary nerves.
n.c.n. Nasociliary nerves.
op.n. Optic nerve.
s.c.n. Short ciliary nerves.



KERMIT CHRISTENSEN—SYMPATHETIC AND PARASYMPATHETIC NERVES

UNIV. OF MICH.



KERMIT CHRISTENSEN—SYMPATHETIC AND PARASYMPATHETIC NERVES

UNIV. OF MICH.

MICROSCOPICAL INVESTIGATION OF THE INNERVATION OF THE TOOTH AND ITS SURROUNDINGS

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The microscopical investigation of the innervation of the tooth certainly is one of the most difficult subjects in histology, and the central question "Can we find nerve fibres in the dentine itself?" has been the subject of prolonged scientific discussion and has been answered in many ways. This central problem of nerve fibres in the dentine is commonly considered identical with the question of how sensations—usually painful—are conducted through the dentine to the trigeminal nerves present in the pulp cavity.

To approach this problem I have, over a period of many years, tried out various methods of silver impregnation technique. Now it is not difficult to impregnate fibres in the dentinal tubules, but before definitely classing these as being nervous, I wanted to see such dentine fibres coming from fibres in the pulp, as this would leave no doubt concerning their nervous character. After years of trying I succeeded in finding in a Bielschowsky-Gros section of human material a number of fibres which were satisfying from the above-mentioned point of view (Plate I, fig. 1). A fibre, which may doubtless be called nervous, is seen rising from the pulp, taking part in the formation of a nervous network situated between the cell bodies of the odontoblasts, and then forming part of a supra-odontoblastic, i.e. infradentinal, reticulum. From this reticulum a number of fine non-medullated fibres enter into a series of adjacent tubules, where they can be followed for some distance into the darkly stained dentine, and finally disappear from sight. Because of the very rough treatment these sections had to stand, the dentine had shrunk away from the pulp, and fibres of Tomes carrying a nervous fibre are seen stretched across this space. After a number of failures with Bielschowsky impregnation and decalcification with NHO_3, and also with de Castro's method (simultaneous fixation and decalcification), I devised another technique, the outcome of which may be judged from the figures in Plate I, figs. 2 and 3, which have been drawn from sections prepared in this way. The dentinal wall of normal, healthy human Canini is ground until it is about 300–500 μ in thickness. This leaves the pulp untouched and the cavity closed. Subsequently, the remaining part of the tooth is sawn into rings (not decalcified), and from these rings I made cross-sections of about 40 μ in thickness on the freezing microtome. These are then impregnated

according to the Gros method, in which, naturally, the normal undecalcified dentine absorbs the silver very avidly. Though not elegant, this way proved to be the most efficient.

Plate I, fig. 2, drawn from a section so prepared, shows a number of nerve fibres rising from the pulp, forming a reticulum between the cell bodies of the odontoblasts, from which many fibrils go over on the fibres of Tomes, and on which they can be followed for some distance. One of these fibrils has a very clear loop-shaped ending, thus once more proving its nervous character, and demonstrating the presence and form of nerve endings in the dentine.

I have never found nervous fibrils outside the dentinal tubuli, so that I am inclined to believe in an exclusively intratubular position; further, as far as I can see, the nerve fibres are embedded in the outer zone of the fibres of Tomes, which latter, in my opinion, completely fill the lumen of the dentinal tubuli.

COURSE OF THE NERVE FIBRES IN THE DENTAL PULP

Stout bundles of nerves enter the pulp, accompanying the larger vessels, and forming an almost complete mantle round the arteries. Medullated fibres separate from the vessels soon after entering the pulp through the apical foramen, branch off at right angles, split up under the layer of odontoblasts—meantime losing their medullary sheaths—and form a wide-meshed plexus. Further, as this branching off takes place repeatedly, a continuous sub-odontoblastic plexus is formed. From this plexus numberless non-myelinated fibrils rise between the cell bodies of the odontoblasts forming a plexus between them. Together with the fibres of Tomes countless nerve fibrils leaving this plexus enter the dentinal tubuli, in which, sooner or later, they terminate.

As far as I can see, the exclusive task of these medullated pulp fibres is to build these sub- and supra-odontoblastic networks, which, in their turn, supply the dentinal nerves. I do not believe in nerve endings in the pulp itself, or round the bodies of the odontoblasts; neither do I believe in non-sympathetic nerve cells in the pulp (Mummery, Münich, Montfort), for I have never seen them, though I followed hundreds of fibrils as far as the network between the odontoblasts.

Along with the vessels, and generally adhering to them, we also have non-myelinated fibrils entering the pulp; on the other hand, it is not uncommon for them to leave the vessels, winding freely through the ground substance of the pulp, and then crossing from one vessel to another. In the wall of the pulp arteries I found a reticulum, built up from extremely fine non-myelinated nerve fibrils, probably situated in the layer of smooth-muscle cells, which I assume to be an efferent (vaso-motor) reticulum. I have never observed anything compelling me to believe that non-myelinated nerve fibrils take part in building the sub-odontoblastic reticulum, or that they have any field of action other than the walls of the blood vessels.

THE PERIODONTAL MEMBRANE

The innervation of the periodontal membrane has been studied in many horizontally cut series from the mandible of young mice, impregnated according to the de Castro-Somnifen technique. Coming from the alveolar bone, a considerable number of nerve fibres for the periodontal membrane reach the same as heavy myelinated fibres. Indeed, I often formed the impression that this was perhaps the case with the greater part of the fibres. In the membrane we can follow the nerve bundles lying close to the alveolar bone, often situated in furrows on its surface (Plate II, fig. 3). In addition to these, others come from the bottom of the alveolus, all of which ascend together in the direction of the gums. The myelinated periodontal nerve fibres showed three different kinds of endings:

(1) Small end-rings, completely surrounded by their periterminal reticulum, are found very close to the bony alveolus, lying on bundles of collagenous (Plate II, figs. 1, *a* and 2) fibrils. This position of the end-rings is not merely accidental, as I concluded from the fact that I discovered three of this type of perfectly similar structure, and all situated as described above, viz. on collagenous fibrils and very close to the alveolar wall (Plate II, fig. 2), whereas I never found them anywhere else in the periodontal membrane. These end-rings belong to medullated axons which do not originate from a plexus, and therefore have an individual innervation.

(2) Terminal reticula surround the nuclei of connective tissue in the peripheral layers of the periodontal membrane. In the material in question the connective tissue had not yet developed beyond the reticular stage. The significance of these terminal reticula escapes me altogether.

(3) The axons, appearing in more centrally situated parts of the periodontal membrane, lose their sheath of myelin and help to build up a reticulum, which is especially wide-meshed in a radial direction (Plate II, fig. 3). Fibrils, coming from this reticulum, penetrate into the layer of cementoblasts, which we find next to the dentine, where they—i.e. the fibrils—have a direction perpendicular to the surface of the dentine. I have often succeeded in following such fibres from the periodontal membrane penetrating radially into the dentine, where they are situated in the dentinal tubuli forming the layer of Tomes near the surface of the dentine of the root. They pass the layer of Tomes and can be followed over a distance equal to about four times its thickness into the central part of the dentine, where they end with exceedingly minute rings. These last-mentioned fibrils exactly follow the terminal branchings of the fibres of Tomes. As these terminal branchings of the protoplasmatic dentine fibres are intimately intertwined in the layer of Tomes, it would appear highly probable that they form a nervous plexus there (Plate II, fig. 3). Though I dare not be absolutely definite as to the existence of this plexus, I nevertheless venture to think this highly probable, and eventually we shall have to bear it in mind when considering our results.

Though not exactly bearing upon the subject in question, it may be of some interest to know that in many of these de Castro-Somnifen sections very considerable numbers of odontoblasts show a well-developed Golgi network. It looks like a minute black sponge, much smaller than the nucleus, situated in the cytoplasm between nucleus and praedentine. (In the young teeth in question the formation of the dentine is in full swing and the layer of dentine still very thin.) As far as I know, Golgi networks in odontoblasts have not yet been described.

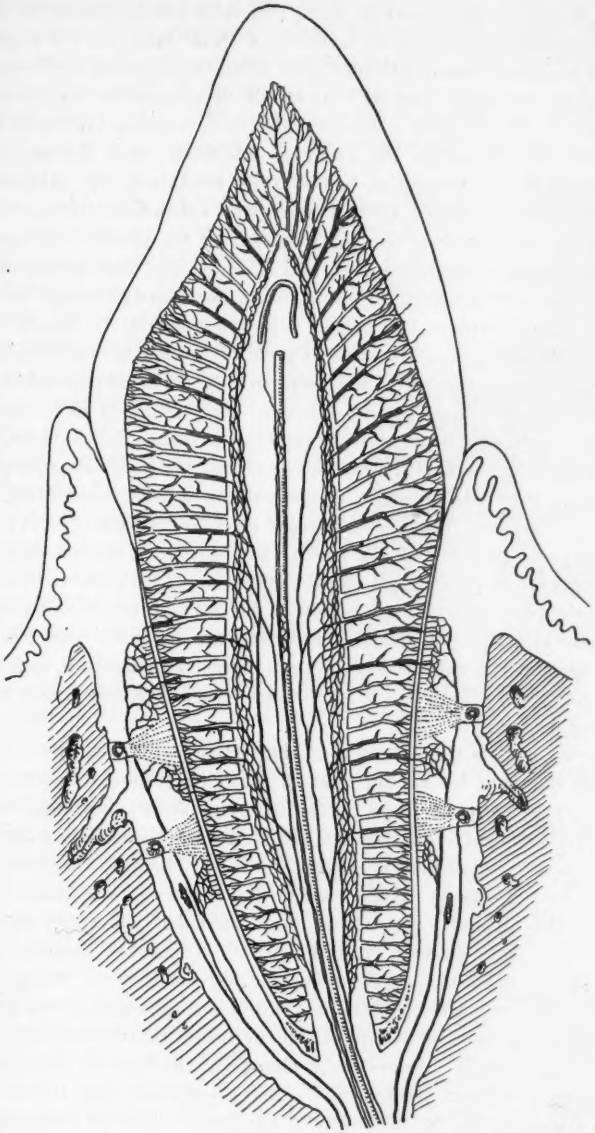
SCHEME OF OBSERVATIONS

Before trying to piece the facts together and to interpret them from a common point of view, I wish to insert a picture of a schematic longitudinal section through a tooth, embodying the several observations. The illustration is not totally symmetrical, in as far as the left side sums up my microscopical findings, whereas the right side shows the conclusions which, in my opinion, can be drawn therefrom. Both halves of the pulp and the periodontal membrane are fully symmetrical, which means that I cannot find any reason why our knowledge should require extension in this field. As far as the dentine is concerned, the number of end-rings situated in the dentinal tubuli has been multiplied, and I have drawn them at different points in the tubuli (near to and farther away from the pulp). I feel justified in doing so because of my numerous observations of nerve fibres in all different parts of the tubuli in any given part of the dentine. I have refrained from drawing nerve fibrils penetrating into the enamel; nevertheless I am of opinion that it is quite possible, or even probable, that they do so. The reason is that I agree with the modern view that many protoplasmatic fibres of Tomes enter the enamel, and that I expect nerve fibrils will be found along the full length of the fibre of Tomes.

FUNCTIONAL CONSIDERATIONS

The functions of these nerves most frequently suggested are: perception of temperature, trophic function, and perception of pain. Perhaps the dentinal nerves subserve one or all of these, or perhaps even additional functions. For the purpose of discussion it seems proper to approach this problem from the point of view of the use of the teeth in the seizure and mastication of food. With this in mind there are two objections to the opinion that dentinal nerves should be engaged in perception of temperature brought into the oral cavity, viz. (1) both the inside and the outside of the mouth have plenty of opportunity of doing this (lips and tongue), and (2) except in civilised life warnings against abnormal temperatures of meat and drink can hardly be of any importance.

Trophic function could readily be imagined, but since the occurrence of purely trophic nerves is such a very debatable matter, this assumption would seem a too easy way out of the difficulties. And exactly the same holds good for the perception of pain. In this connection we should do well to keep in



Text-fig. 1. Scheme of the neurological relations within and around the tooth. Left half: the results obtained; right half: my conclusions from these findings.

mind the fact that though experimental stimulation of certain nerves may be perceived in our consciousness as pain, this does not authorise us to assume that the exclusive physiological function of those same nerves is perception of pain. A too ready assumption of pain as an explanation of the presence of intradental nerves might lead us to overlook other possible functions.

In regard to the function of the periodontal membrane end-rings I want to put forward the following: the end-ring lies flatly on a collagenous tissue bundle, which latter, in repose, is not quite stretched, but takes a slightly undulating course (cf. Häupl, 1931, Kantorowicz's *Handbuch*, Bd. III, S. 2000–4). As a result of movements of the tooth caused by the pressure of chewing, the collagenous bundle is stretched taut, and the end-ring, fixed on its substratum by means of its periterminal network, must necessarily change its form. It is this change of form of the ending which I consider to be the adequate stimulus for the nerve. Consequently, I suggest that the task of these end-rings lies in the perception of pressure, not because they are being pressed themselves, but because the end-rings get stretched in their plane of extension. Every movement of the tooth in the alveolus, and any pressure, in whatever direction, brought to bear on the tooth, stretches a special group of collagenous bundles in accordance with the direction of the pressure, thus stimulating a special group of end-organs. These end-organs have individual innervation, and consequently any pressure on the tooth will stimulate a special corresponding group of nerves, and accordingly of cells in the Ganglion Gasseri. The direction of the pressure determines the location of the nerve fibres that will be stimulated, and the stimulus will probably be proportionate to the degree of pressure. In doing so, the central nervous system perceives direction and degree of pressure, so that eventually reflex mechanisms can be put into action to regulate the chewing pressure.

And now, what is the function of the nerve endings in the dentine? To answer this question we should bear in mind that their diameter measures about $0{\cdot}5\,\mu$, that they are fixed on the fibres of Tomes, and that they occur in large numbers scattered through the dentine. Naturally, a definite and exact answer to this question is lacking, but to us the following interpretation appears to be reasonable. Even though the tooth has a certain amount of play within the alveolus, every time it is touched it will suffer deformation, however slight. The dentine contains an immeasurable number of tubuli, each completely filled by a protoplasmatic fibre of Tomes. Many, if not all, fibres of Tomes carry a nerve fibril, ending in a ring, which latter lies fixed in the protoplasm of the fibre of Tomes. The deformation of the dentine (through touching) is necessarily followed by a change in the form of the cross-section of the dentinal tubulus (increased diameter perpendicular to the pressure, decreased diameter in the direction of pressure). This in its turn must be followed by a deformation of the fibre of Tomes (completely filling the lumen of the tubulus), and consequently by deformation of the fixed end-ring of the dentinal nerve. Thus even the slightest deformation of the dentine results in

minimal changes of form of the very delicate end-rings, which may very well represent the adequate stimulus for the nerves.

Deformation of the dentine need not exclusively be caused by touch or collision, as frictional heat (resulting from chewing) will have the same deforming effect on dentine, tubuli, fibres and end-rings.

Another possibility is that chewing causes some kind of vibration in the tooth, giving rise to deformation of the dentine and ultimately to nervous stimulation.

Finally, and for the sake of completeness, I wish to mention that chewing must generate frictional electricity, which will want to flow along the tooth, perhaps forming a stimulus for the dentinal nerve. The mechanism of such electrical stimulation, however, is unknown to me, as also its biological significance.

Ignoring for a moment this last type of stimulation, we have found periodontal and dentinal nerve endings, similar terminal organs, which we suggest are responsive to changes of form: displacement of the tooth stimulates the periodontal end-rings, individually innervated; deformation of the tooth stimulates the dentinal end-rings, diffusely innervated (i.e. innervated from a network).

But whatever the function of the dentinal end-rings may be, it certainly seems incorrect to speak of the innervation of the odontoblasts or to look on the odontoblasts as neuro-sensory cells or to compare the pulp nerves with the optical nerve. The task of the odontoblasts in the innervation of the tooth is to accommodate the nerve endings, they having themselves no sensory function.

The number of nerve fibres and endings in the dentine being very large, it may be inferred that they form an essential factor in the functioning of the tooth. We might perhaps regard the nerve terminals of the tooth—thanks to the manner of the tooth's fixation in the jaw—capable of regulating by reflexes the motion and the course of the chewing act.

It seems not unreasonable to suppose that in the chewing act two different functions are subserved by these two groups of terminal organs. On the one hand, we may suppose that moving the tooth in the alveolus (stimulation of periodontal endings) is always accompanied by deformation of the tooth (stimulation of dentinal endings); on the other hand, deformation of the tooth might occur independently, i.e. without movements of the tooth. In the first instance (movement plus deformation) it would seem possible that the two groups of endings in combination would take care of the reflex mechanisms required by the different qualities of the chewing pressure. Further, one might readily think that dentinal endings were excited by deformation according to nature and location of material round the teeth, long before chewing pressure or stretching of periodontal collagenous bundles occur. This would mean that before chewing actually begins, harmonious co-operation of the masticatory muscles is reflexly ensured. In general it might be said that the

dentinal endings prepare for the chewing act while the periodontal endings regulate it during its execution. One is of course speaking of extremely delicate stimuli, and of reflexes, outside the sphere of consciousness.

SUMMARY

1. From sections of human and mammalian teeth, prepared according to methods of Bielschowsky, de Castro and Gros, it is demonstrated that pulp nerves, after having formed a network between the odontoblasts, enter the dentine. In the dentine they take an intratubular course, situated on, or more probably embedded in, the fibres of Tomes, where they end in delicate rings (Plate I, figs. 1 and 2). It is believed that all the myelinated pulp nerves terminate in the above-mentioned intradentinal endings. The odontoblasts are not "innervated", and are not to be regarded as sensory cells.

2. Non-myelinated pulp fibres form probably an efferent reticulum in the wall of the blood vessels; repeatedly they freely cross the ground substance, going from one vessel to another. There is no evidence that the non-medullated pulp fibres have any other function than innervation of the vascular walls.

3. In the periodontal membrane we found: (*a*) end-rings, provided with a periterminal network, lying flatly on collagenous bundles, individually innervated, situated near to the alveolar wall (Plate II, figs. 1, 2 and 3), (*b*) terminal networks round connective tissue nuclei, and (*c*) a nervous network from which nerve fibres go over into the dentine, to end with very delicate rings inside the dentinal tubuli.

The periodontal endings are probably fixed on their substratum by means of the periterminal network, and are adequately stimulated by the change of form they undergo when the underlying collagenous bundles are stretched taut by the pressure of chewing. Very probably these endings represent the receptor organs of reflex arcs which come into action at the moment the opposite teeth touch. It is suggested that the intradentinal endings get their adequate stimuli in just the same way as the periodontal end-rings, viz. by the changes of form in the dentine. Thus the intradentinal endings collaborate with the periodontal end-rings in supplying the necessary stimuli for the reflex regulation of the chewing mechanism.

The tooth might be regarded as an organ, fitted on the one hand for the function of chewing and on the other by its intradental and extradental nerve endings, which are stimulated by deformation and movement of the tooth, to act as the receptor in the reflex arcs for the regulation of the act of mastication.

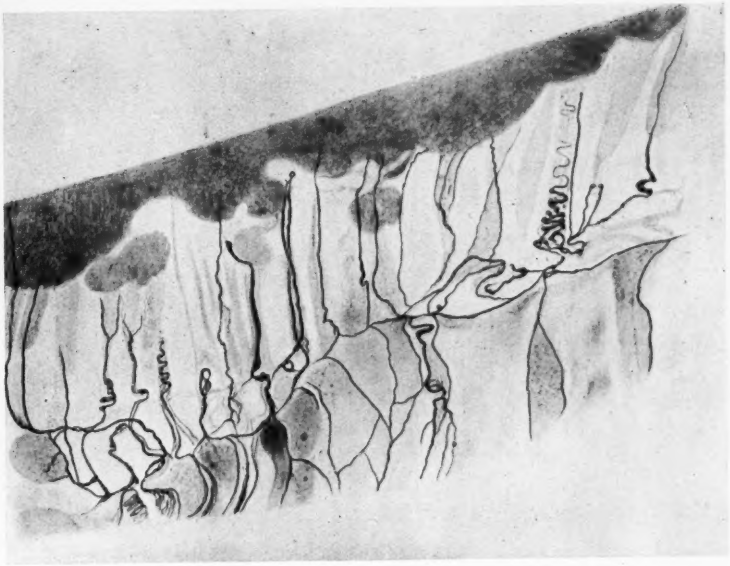


Fig. 2

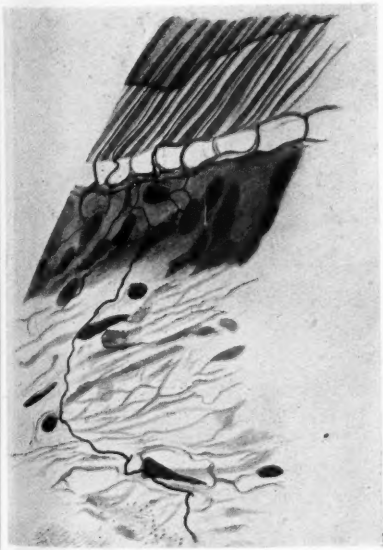
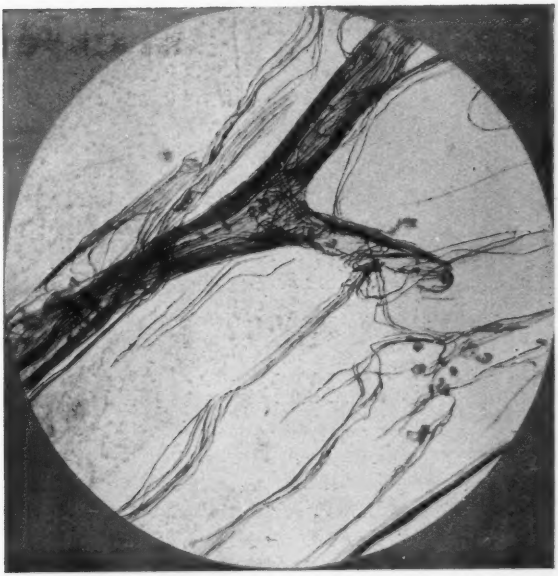


Fig. 1



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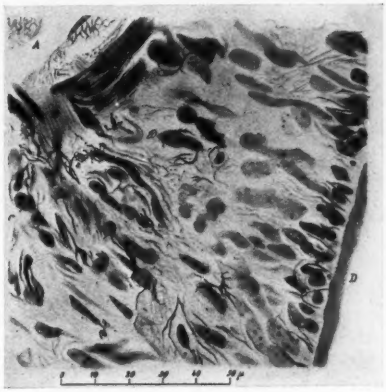

Fig. 1

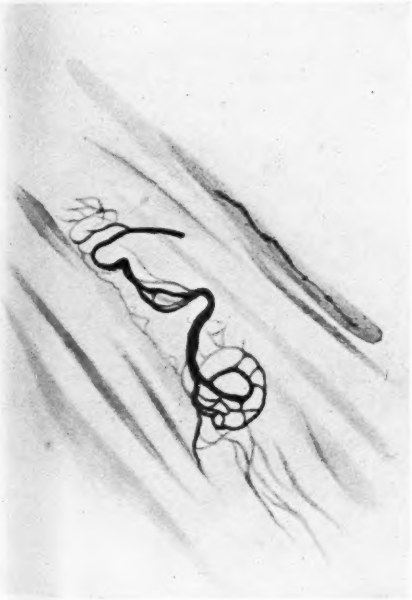

Fig. 2

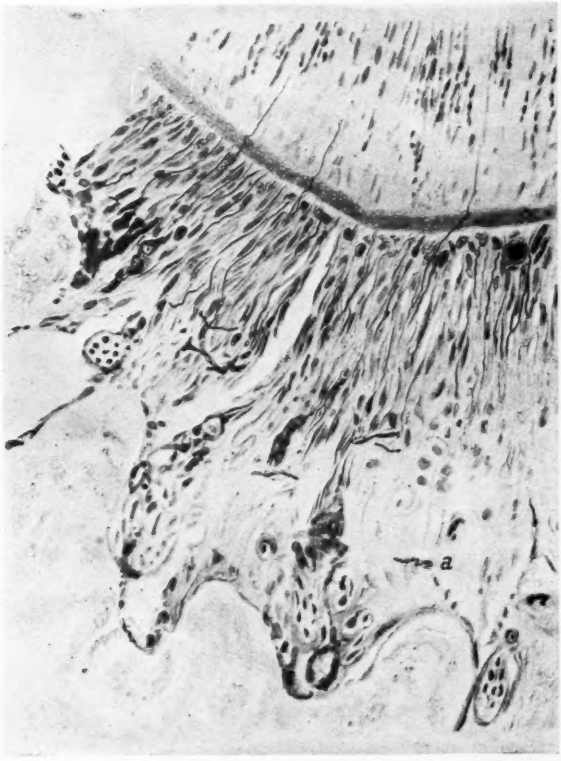

Fig. 3

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Nerve fibre ascends from the pulp cavity, forms a network between the cell bodies of the odontoblasts, and enters the tubuli of the dentine. Neck of a human premolar. Bielschowsky-Gros method.

Fig. 2. Pulp nerves form a network between and on top of the odontoblasts and enter the dentinal tubuli. One of them ends in a nerve end-ring situated on the fibre of Tomes. Neck of a human premolar. Bielschowsky-Gros method.

Fig. 3. Myelinated and non-myelinated nerve fibres around the wall of a pulp vessel. Some of these non-myelinated nerve fibres swing through the ground substance of the pulp. Human pulp. Gros method.

PLATE II

Fig. 1. Periodontium. Mouse. de Castro-Somnifen method. A. Alveolar bone. B. Collagenous bundle on which lies a nerve end-ring with periterminal network (*a*). D. Dentine with dark layer of Tomes. Between the cementoblasts fibres are seen running perpendicularly to the surface of the dentine. Mag. 1100.

Fig. 2. Nerve end-ring with periterminal network fixed on a collagenous bundle of the periodontium. Mouse. de Castro-Somnifen method. Mag. 3000.

Fig. 3. Mouse. Periodontium. de Castro-Somnifen method. Myelinated nerve bundles situated in furrows of the alveolar bone, cut across. *a*, nerve end-ring on a collagenous bundle. Nerve fibres run from the periodontium into the dentine, where they end in minute end-rings lying in the dentinal tubules.

INVESTIGATIONS ON THE PARS INTERMEDIA OF THE HYPOPHYSIS IN ANTHROPOID APES AND MAN

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The hypophysis is recognised more and more as a kind of central organ for the internal secretion. The number of hormonic actions ascribed to it is growing from year to year. Attempts are made to connect definite hormonic actions with definite portions of the hypophysis. On the other hand, the morphological structure of this organ is by no means fully known. One of the much-debated questions concerns the so-called pars intermedia which is situated between the anterior and posterior lobes.

For many years a discussion has been going on concerning the existence, character and importance of this small lobe in the adult human (see previous papers). It may be possible that the comparative anatomy, especially by studying animals that are the nearest akin to Man, will extend our knowledge on this subject. At the same time, light might be thrown on the problem of the relation between Man, apes and other Mammals.

MATERIAL AND METHODS

Hypophyses of anthropoid apes are not easily obtained. In 1922, the Zoological Garden of Hamburg, Germany, gave us the hypophysis of one chimpanzee. Through the kindness of the firm of Hagenbeck, we were enabled to examine the hypophysis of an adult orang-utan in 1928. Dr Howard Fox gave us a number of hypophyses from the large material of the Zoological Garden in Philadelphia. Unfortunately, not all of these could be used for our purpose. The material of this paper consists of three hypophyses from the *Cebus* family, designated as *Cebus capucinus*, *Cebus capillatus* and *Lagothrix lagotricha* (woolly monkey), two orang-utans (*Simia satyrus*), one chimpanzee (*Pan niger*), and one gibbon (*Hylobates*).

The hypophyses were embedded in paraffin and cut serially at a thickness of 10 μ. The sections were stained with haematoxylin and eosin. Contour drawings were made with a Zeiss micro-projection apparatus. The image is projected on the drawing bench by means of a mirror which is fastened to the tube of the microscope. At the left of the person who does the drawing an ordinary microscope is standing on the bench to permit continuous control of the drawing at a higher magnification. Standing at a high bench (36·5 in.), one can rapidly shift from the drawing machine to the ordinary microscope and work for a long period without undue strain.

The contours of the lobes were drawn on Bristol paper. The contour sketches were then cut out with scissors and weighed. The method thus, in all essentials, follows that developed by Hammar for the quantitative study of the thymus and applied to the hypophysis by Rasmussen and Herrick (1922). Before turning to the method of cutting out with scissors, I also tried the planimeter. I agree with C. M. Jackson that the scissor method is to be preferred.

The accuracy of the method was tested in the following way. Two contour drawings chosen at random, the one 2 cm. diameter, the other one 8×3 cm., were drawn ten times each on the Bristol paper, then cut out and weighed. The mean error of the weighings was 0·4 per cent. For its determination, the formula $\frac{\Sigma (+v)}{n \sqrt{n}}$ was used, Σ standing for the sum of deviations, v and n for the number of weighings. By this control, all sources of error seem to be accounted for. In the fear of being misled by compensation of errors, further controls were made. Pieces of paper were weighed which were exactly alike in size and a mean error of 0·25 per cent. was found, using the above-mentioned formula. The mean error caused by the process of drawing and cutting, therefore, must be less than 0·2 per cent. This appears incredibly low for as coarse a method as drawing along a hand-drawn pencil line. Most of the many small deviations of pencil and scissors probably compensate each other.

When the same piece of paper was weighed on different days, the results became irregular, because the humidity changes in the atmosphere of New York City are sudden and strong. The cut-out papers in the vacuum were therefore dried; but this led to uneven absorption of moisture by the dried paper before and during the procedure of weighing, and again did not give constant results. The difficulties were finally overcome by leaving all papers belonging to one series freely exposed to the air of the weighing room for 24 hours before the weighing.

FINDINGS

In the three monkeys from the *Cebus* family the pars intermedia was large. The picture was similar to the well-known illustration given by Herring many years ago. In the *Cebus capillatus*, the pars intermedia, including the pars tuberalis, had one-tenth of the volume of the anterior lobe, in the capucine monkey one-fourth; in the hypophysis of the woolly monkey exact measurements could not be taken, but it was evident that the pars intermedia was well developed.

The findings in the apes were strikingly different. In the old male orang-utan from Hagenbeck, the pars intermedia was so small that its contour drawings could not be cut out and weighed. Complete serial sections were available. The volume of this pars intermedia was only a small fraction of 1 per cent. of the volume of the anterior lobe. The orang-utan from the Philadelphia Zoological Garden had a larger pars intermedia. It was possible to cut along the pars intermedia and to weigh the minute pieces of paper.

Still, the volume of this pars intermedia was only 0·5 per cent. of that of the anterior lobe. Of the chimpanzee hypophysis, many sections from different levels were available (no complete series). They definitely indicated that the pars intermedia was very small, in the same order of magnitude as that of the orang-utan. The hypophysis of the gibbon was partly torn. While accurate figures for the volume of the pars intermedia of the gibbon cannot therefore be given its smallness could be satisfactorily recognised.

I have not been able, so far, to secure the hypophysis of a gorilla.

Detailed descriptions of the hypophysis of the chimpanzee and the orang-utan have been given in previous publications. The orang-utan from Philadelphia had died from ulcerous colitis and secondary anaemia (Philadelphia Zoological Garden, No. 4992). The hypophysis was complete. The anterior lobe measured $8 \times 5 \times 4$ mm., the posterior lobe $6 \times 4 \times 3$ mm. The available portion of the stalk was 4 mm. long, 1·5 mm. thick (measurements taken after long fixation in formalin). In most of the slides, anterior lobe and posterior lobe touch each other. The posterior portions of the anterior lobe contain much connective tissue as often found in old humans also. The anterior lobe partly encircles the posterior lobe. The pars intermedia which, as mentioned, is very small, in the sections gives the picture of a sickle. The stroma of the pars intermedia is red in the van Gieson stain, while that of the neighbouring anterior lobe is brownish yellow. This staining reaction of the connective tissue also is often seen in the hypophysis of old humans. The nuclei in the pars intermedia are more deeply stained than those in the anterior lobe.

In these series, the pars intermedia extended through eighty sections. This method of cutting out and weighing was applied to these eighty sections only. In the remainder of the series the sections were counted for each slide, and the average size of the anterior lobe was determined for each slide. With the smallness of the pars intermedia and the large size of the anterior lobe, this simplification of the method did not influence the result to any appreciable extent.

The hypophysis of the gibbon (*Hylobates*) from the Zoological Garden, Philadelphia, measured $6 \times 3 \times 1{\cdot}7$ mm.; the available piece of pedicle was 4 mm. long. The posterior lobe could not be recognised with the naked eye. The study of the five hundred sections clearly showed that the pars intermedia was very small. An exact determination of its size was impossible because the specimen was torn. But even under the assumption that the torn portions contained much more pars intermedia substance than the remainder of the sections, the highest possible figure for the volume of the pars intermedia remained very small, probably near the value found in the chimpanzee.

In a 55-cm. long new-born girl I found the volume of the pars intermedia 1/135 of that of the anterior lobe. This figure is nearer to the figures found in the adult apes than to the values found in the monkeys. Covell gives a ratio of 1:42 for the new-born human. The figures given by Atwell and Woodwarth represent a ratio of 1:64. Rasmussen's (1928) figure for the new-born is 1:40.

Such a high degree of variation in the figures may in part be due to the variation in the size of the pars intermedia. But there are other factors involved. One sometimes has doubts whether a certain area belongs to the pars intermedia or not. It is not always clear from the reports in the literature whether or not the pars tuberalis has been considered part of the pars intermedia or not. Since in our specimen from the capucine monkey, for instance, the pars tuberalis had one-quarter the volume of the pars intermedia, this may account for differences in the figures of different authors. Atwell and Woodwarth have found still higher values for the pars tuberalis in human infants and in female cats. Another source of error lies in the varying spaces occupied by blood vessels and gland-like structures, according to their being either distended or collapsed.

DISCUSSION AND COMMENT

These investigations prove that the adult orang-utan and chimpanzee have as rudimentary a pars intermedia as the adult human. It is made highly probable that the gibbon behaves in similar fashion.

The smallness of the pars intermedia in the adult human has been stressed repeatedly, but still is not sufficiently recognised. As Rasmussen puts it: "Some writers lack a concrete idea of the actual magnitude of the tissue known as the intermediate portion of the human hypophysis." This statement, one regrets to state, is true, even for articles printed in 1935. According to Rasmussen's latest measurements, the pars intermedia of an apparently normal female adult may weigh as little as 0·3 mg. This is only one-half of 1 per cent. of the weight of the parathyroid glands. Including the colloid it generally represents only 1·5 per cent. of the total hypophysis in the human adult. In addition, it is exceedingly variable, having a coefficient of variation of over 100.

From the foregoing, it is evident that I do believe in the existence of a rudimentary pars intermedia in Man. Not all authors share this opinion. I do not believe, however, that the pars intermedia of Man and apes represents a true functional equivalent of the pars intermedia of other Mammals. Aside from the anatomical and zoological interest, the comparative study of the pars intermedia is important for our conception of the source of hypophysial hormones. If Man and apes have no functioning pars intermedia, then the hormones which other animals produce in the pars intermedia must be produced elsewhere by Man and apes, obviously in the anterior lobe. As far as our knowledge goes, nobody has ever worked with extracts of apes' hypophyses, and no attempts have been made to prepare a separate extract of the human pars intermedia. But the literature contains very little even about extracts of the two main lobes of the human hypophysis. Walter Lampe, who in 1926 made potent extracts from a number of human hypophyses, does not discuss the structural difference between the animal hypophyses generally used for extracts and the human hypophysis. Lampe states that it was easy to separate the three lobes. I consider it almost impossible to separate the anterior lobe from

the rudiments of the pars intermedia in the adult human. With such difficulties forbidding experimental approach, one might try to clear up some aspects of the problem by morphological study.

Aschoff, while sharing the opinion that the pars intermedia in Man cannot be identified morphologically with that of other Mammals, does nevertheless believe in a function of the pars intermedia in Man. In my opinion, this zone in Man and apes does not bear the earmarks of a functioning organ. Normal organs of internal secretion, no matter how small they may be, do not show as much variation as does the pars intermedia in Man and apes.

It is an analogous consideration which militates against the widespread idea that the migration of epithelial cells into the posterior lobe represents the basis of a normal function. These cells are believed to form a connection between the actively secreting epithelial portions and the neuroglia-like tissue of the posterior lobe and the stalk. But experienced observers agree on the irregularity of this cellular migration. In some human hypophyses, the posterior lobe hardly contains any epithelial cells; in others, large compact epithelial masses anatomically simulate a tumour without there having been any evidence of altered hypophysial activity during life. This migration of epithelial cells into the posterior lobe is an extremely interesting process, perhaps a unique one. But it does not appear to be the basis of a normal function. The epithelial cells migrate, they finally disintegrate, and hyaline-like bodies and pigment are formed. The cell migration also seems to lead to destruction of posterior lobe tissue. Posterior lobes which contain large amounts of epithelial tissue are not necessarily enlarged, and the remaining normal tissue does not appear compressed. Therefore, some of the posterior lobe tissue must have been destroyed. Such destruction of tissue, in my opinion, does not denote normal function.

Extensive lesions of an incretory organ may be present without disturbance of function. This is generally known, and it is explained by the fact that a small amount of intact tissue may be sufficient for normal function. One might, therefore, argue that the lack of functional disorders in people whose hypophyses exhibit irregularities in the region of the pars intermedia does not prove the physiological insignificance of the pars intermedia. The situation, however, is a little different. We sometimes find large colloid cysts at the borderline of the two main lobes. In these hypophyses, all the possibly existing pars intermedia tissue must have been exposed to strong pressure and that for a long time, since the cysts contain very thick material. Nevertheless, no hypophysial or other incretory abnormalities have been observed in such patients. Thus the tissue in the pars intermedia region cannot be necessary for an important function. If, on the other hand, the functions of the pars intermedia in Man and apes have been taken over by the large anterior lobe, then the lack of disturbance, in spite of the distinct anatomical change in this region, is readily understood because enough anterior lobe tissue is left for carrying on the normal functions.

A further difference between the pars intermedia of other Mammals and that of Man and apes lies in its topographical relations to the other lobes. In most Mammals, the pars intermedia is intimately connected with the posterior lobe and sharply separated from the anterior lobe, except for some subcapsular portions ("Umschlagsteil"). In Man and apes, on the contrary, anterior lobe and pars intermedia are so closely connected that it is often difficult, even under the microscope, to establish a borderline. A kind of connection with the posterior lobe is represented mainly by the inconstant migration of epithelial cells as far as these really come from the pars intermedia. If then, as some authors maintain, the peculiar structure of this region is indicative of a peculiar function, we must, in the face of such anatomical differences, not expect the same function.

The thesis that in Man and the ape the intermediate lobe does not function as in other animals seems of more importance than the question whether or not the structures which are present in Man and the ape should be labelled pars intermedia or not.

A full understanding of the hypophysial apparatus will be reached only when we learn more about the posterior lobe. This lobe is highly constant from Amphibia to Man; thus it must be important. The complicated nervous structures of the posterior lobe, as demonstrated by modern histological methods, cannot be explained as "supporting tissue". There is nothing to be supported. The stalk, being cylindrical, may appear fitted for the functions of receiving and conducting the products of the anterior lobe, but it would not do to ascribe conducting functions to an ellipsoid, capsulated organ like the posterior lobe. The posterior lobe of hypophysis may be the seat of highly specialised, little known functions which somehow are intermediate between secretory and nervous activity. Such a concept does not seem as hypothetical to-day as it might have appeared only a few years ago. The hypothalamic region itself harbours structures which obviously are secreting (droplet formation, close relation between cells and capillaries). Such structures have been found in Man and in animals (Scharrer, Gaupp). In the light of these findings, our ideas concerning the hypophysis-diencephalon apparatus will have to be revised.[1]

It is dangerous to use morphological peculiarities for drawing conclusions as to altered function. We must realise that the multiformity of the animal kingdom is caused to a great extent by variations whose possible functional importance is beyond our comprehension. Could the pars intermedia of Man and the ape perhaps represent such a variation caused by coupling of genetic factors, for instance, without any relation to function? I do not think so.

[1] In this connection it is highly interesting that the hypothalamus itself undergoes a phylogenetic reduction in Mammals as does the pars intermedia of hypophysis. This part of the diencephalon is large in Marsupials and Insectivores, smaller in Rodents and Carnivores, still smaller in the monkeys, very small in orang-utan and gorilla, and smallest in chimpanzee and Man (Grünthal).

The reduction of the pars intermedia in volume, as well as in differentiation, is so persistent throughout phylogenesis from Fish to Man that one feels logically compelled to postulate corresponding changes in function.

The more one sees hypophyses of different species, the more important appears the creating of a complete comparative anatomy of this organ. We should know whether or not animals with one-sided differentiation, notably of body proportions, have strikingly different hypophyses. On the other hand, we must find out if marked differences in the hypophyses of animals exist while these animals are highly similar to each other. A conspicuous example has been given by Wislocki. The small porpoise (*Tursiops truncatus*), a member of the whale family, certainly has queer shape and proportions considering that it is a Mammal, and its hypophysis has been found by Wislocki to be distinctly different from that of other Mammals. Its two main lobes are separated from each other by a fold of the dura mater. The pars tuberalis in this hypophysis is the only glandular tissue in contact with the nervous system.

In the large blue whale (*Balaenoptera sibbaldii*), according to Valsö, the two main lobes are separate and only connected with each other by a thin pedicle. Thus these two whales have hypophyses which are entirely different from each other and from the hypophyses of any other species. The literature contains very little information about the hypophyses of whales.[1]

The question arises, how many different types of hypophyses exist in the whale family? It would be important to know the hypophyses of the sea cows (*Sirenia*). These aquatic Mammals in shape and body organisation resemble the whales, but they are widely separated from them zoologically. A similar investigation could be made on the hypophysis of seals and other Pinnipeds in comparison with the well-studied hypophysis of the related land Carnivores. The possible phylogenetic, species-forming, influence of internal secretion may be approached by this route. We also need a comparative study of the migration of epithelial cells into the posterior lobe. This unique process has not received the attention it deserves. The posterior lobe of almost every human adult, of many children and of some new-borns, contains epithelial cells resembling the basophilic cells of the anterior lobe. These cells are not carried passively by some current of fluid; they obviously migrate by themselves, a process hardly paralleled in post-embryonic life.[2]

Comparative investigations must show what relation, if any, exists between this cell migration and the phylogenetic reduction of the pars intermedia. Rat, rabbit and cow have no migrating epithelial cells in the posterior lobe (Berblinger). I could not find any epithelial cells in the posterior lobe of the anthropoid apes (orang-utan and chimpanzee). The gibbon hypophysis was not complete; one, therefore, cannot be sure whether or not there is epithelial

[1] I hope in a later publication to deal with the study of the whale hypophysis and allied general problems.

[2] One might consider the neuro-epithelial elements in the vermiform appendix a parallel.

invasion in the posterior lobe of the gibbon. In two capucine monkeys a little was found, nothing in a *Macacus*, but in a *Cebus capillatus* many epithelial cells were seen in the posterior lobe. A complete series of a dog's hypophysis failed to show invasion of posterior lobe, except for a few epithelial elements directly adjoining the pars intermedia. Many more specimens from different species of all ages must be studied before any conclusions concerning the role of this cell migration may be drawn.

SUMMARY

1. The pars intermedia of hypophysis in chimpanzee, orang-utan and, probably, in the gibbon is rudimentary.

2. In this respect the hypophysis of anthropoid apes resembles that of Man and differs from that of monkeys and other Mammals.

3. It seems highly improbable that the rudimentary pars intermedia of Man and apes should function in the same way as does the well-developed pars intermedia of other Mammals.

4. For a number of reasons it is desirable to have a more complete comparative study of the hypophysis, notably of the pars intermedia.

5. A complete knowledge of the comparative anatomy might throw light on the possible phylogenetic influence of hypophysial changes.

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A PURKINJE CONDUCTION NETWORK IN THE MYOCARDIUM OF THE MAMMALIAN VENTRICLES[1]

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The early literature concerning the anatomy of the conduction system in the mammalian heart deals almost exclusively with the investigation of the structure and architecture of the sino-auricular and auriculo-ventricular nodes, the Bundle of His with its two branches and the subendocardial Purkinje network. Aside from occasional and vague allusions (1, 10, 13) no reference is made to a myocardial Purkinje plexus, i.e. a network of conduction fibres which arises from the subendocardial system to penetrate and ramify through the muscle substance of the ventricles. Recently, however, Whalin (14), in the calf, and Cardwell and Abramson (2), in the ox, have described such structures traversing the interventricular septum and connecting the conduction systems found just beneath both septal endocardial surfaces. The latter workers also found a complex myocardial network in the outer walls of the ventricles. The present communication is concerned with a gross and histologic study of the myocardial Purkinje fibres in the ox, dog, pig and sheep hearts.

METHOD

The conduction system in the sheep and ox was studied by means of the injection method. The hearts were opened and prepared in the manner described by Cardwell and Abramson (2), and the Purkinje system injected either with a 1:25 dilution of India ink or an acetone suspension of red oil paint. In this way the subendocardial and (with repeated injections) the myocardial Purkinje plexuses are demonstrated. Subsequent histologic sections of fresh as well as injected tissues confirmed and supplemented the gross findings.

The conduction system in the dog's heart could not be investigated by means of the injection method because of the relatively small size of the individual Purkinje fibre and its sheath (an observation previously made by other investigators (7)). As a result, histologic examinations alone were utilised. In the case of the pig heart, since the injection mass spread with considerable difficulty, histologic methods were also necessary.

Histologic technique. Bouin's picro-formol and strong Flemming's solution were eventually decided upon as fixatives of choice. Blocks of tissue were

[1] Aided by a grant from the American Association for the Advancement of Science.

generally taken from several places in the septum and the outer walls of both ventricles, each section including the two endocardial surfaces in the case of the former and endocardium and epicardium in the case of the latter.

While the standard haematoxylin and eosin technique was found adequate for the differentiation of the myocardial Purkinje fibres in the sheep, ox and pig heart, this method in the dog did not sufficiently distinguish the fibres from contiguous ventricular muscle. Accordingly, the sections (6–10 μ) were stained by a modification of the rapid Heidenhain haematoxylin method (10 min. in the mordant and 10 min. in the dye, with both at 60° C., followed by differentiation in the iron alum). The slides were then treated with Mallory's triple connective tissue stain. As a rule, better results were obtained by somewhat understaining with acid fuchsin and then relatively overstaining with aniline blue and orange G. Differentiation, which took place rapidly, was followed under the microscope after flooding the stained sections with 95 per cent. alcohol.

RESULTS

Histologically, the myocardial Purkinje fibres of the various species have sufficient features in common to allow for the establishment of certain criteria for their recognition and differentiation from the surrounding myocardium.

Staining reactions. The Mallory triple connective tissue stain consistently gives marked differentiation.[1] The muscle fibre acquires a purplish colour while the myocardial Purkinje fibre has an orange-mauve cast. The nuclei of both are blue-black or red, depending upon the length of the acid fuchsin treatment. The connective tissue is a deep blue. If differentiation is followed under the microscope, brilliant contrasts with a selected Purkinje fibre can be observed.

Fibrils. The fibrils of a Purkinje fibre tend to be distributed at the periphery of the cell in a diffuse fashion as opposed to their compact and more central appearance in the muscle fibre; as a result, the longitudinal striations of the Purkinje cell are more apparent than the transverse. Each fibril is studded with evenly spaced beads which with similar structures of adjacent contiguous fibrils form the transverse striations. The fibrils appear to pass uninterruptedly from one fibre to another wherever two or more of the latter course together through the myocardium.[2] The syncytium thus formed is similar to that of the subendocardial network.

Nuclei. The myocardial Purkinje fibre contains nuclei which tend to be spherical, vesicular and multiple as compared with the oval, granular and

[1] The difference in staining reactions between Purkinje and muscle fibres is probably due to the difference in concentration of intracellular elements, the former having a relatively more abundant and homogeneous cytoplasm.

[2] It is said that the fibrils of the Purkinje fibres ultimately merge with those of ordinary myocardium (Tawara (13), de Witt (4)). While an apparent continuity has been frequently observed between the two, we are not prepared to confirm this statement because of the great opportunity for error, *e.g.* artefact, angle of section, etc.

single nucleus of the muscle fibre. The perinuclear space observed in the subendocardial Purkinje fibre also occurs in the myocardial fibre. It is undoubtedly real, the fibrils being deflected to the periphery of the Purkinje cell, none being found in the vicinity of the nuclei. The emptiness of this space in the stained section is probably an artefact, since it is more pronounced after Bouin's and alcohol fixation than after osmic acid fixation. A somewhat smaller space containing a granular cytoplasm is also present in ordinary myocardium.

Sheath. The connective tissue sheath is one of the most constant characteristics of the conduction network. It accompanies the myocardial Purkinje fibre from its origin as a branch of the subendocardial network, throughout all its extensive ramifications up to the epicardium (Plate I, fig. 1). Its thickness seems to be related to the number of fibres enclosed, so that single and terminal fibres have an extremely thin and delicate sheath. Throughout its course it blends with the mysial connective tissue septa and the adventitia of frequently adjacent blood vessels. The Purkinje fibre appears to lie free within the sheath, an observation which is supported by the ease with which the latter may be injected. Despite careful search, even in preparations in which vascular endothelium was well stained, no cells comparable with the endothelial lining of blood vessels could be made out in the sheath wall except in occasional instances, when the walls were exceptionally thick, as in sites directly below the endocardium and in the immediately adjacent myocardium. Ultimately the sheath appears to merge with the interstitial tissue of the myocardium.

Size of Purkinje fibre. Depending on the species, the myocardial Purkinje fibre varies from two to five times the width of the muscle fibre. Even though structures analogous to the intercalated discs of myocardium are present, so varied is the distribution of the fibrils within the syncytium that it is difficult to define the length of the cell precisely.

Continuity and relation between subendocardial and myocardial Purkinje fibres. In all the species studied serial sections showed direct continuity of the myocardial Purkinje fibres with those lying immediately below the endocardium (Plate I, fig. 1). In no respect are these two divisions morphologically different, and the above criteria apply equally well to both the subendocardial network and to its branches which penetrate the myocardium (Plate I, fig. 2 and Plate II, fig. 1). In general the myocardial fibres leave the subendocardial Purkinje plexus in the form of large bundles or trunks, which very soon begin to subdivide until single fibres result. As might be expected, the nearer to the endocardium the more numerous are the myocardial Purkinje fibres, since those found close to their origin consist of fibres about to terminate locally as well as others destined for the deeper layers of muscle. Consequently, the fibres in the vicinity of the epicardial surface are single and rather sparse. The myocardial trunks of the left ventricle are thicker, i.e. contain more Purkinje fibres, than those of the right ventricle. This seems to be correlated with the mass of ordinary muscle in each ventricular wall.

Blood supply. The blood supply to Purkinje tissue is unquestionably poorer than that to an equivalent mass of ordinary muscle. Capillaries are infrequently seen associated with single Purkinje fibres and are only occasionally present within the septa of the larger bundles.

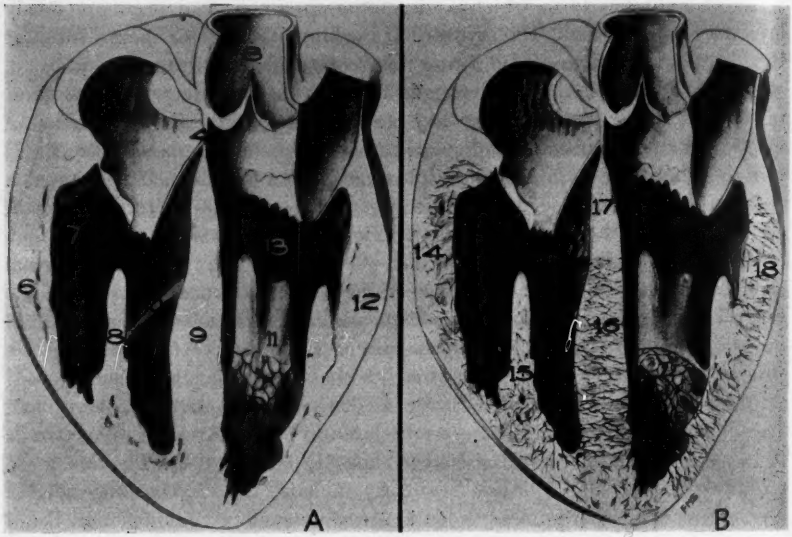
The ventricular myocardial conduction system in the ox heart. The microscopic anatomy of the myocardial Purkinje fibres in the ox heart was studied in a series of eight hearts in which some of the specimens had been previously injected with India ink. The myocardial Purkinje fibre in this species is by far the most highly differentiated, being readily identified with the haematoxylin eosin or the Mallory phosphotungstic stain. It is characterised by its great width (five times that of an ordinary muscle fibre) and diffuse distribution of fibrilli, which appear few in number in relation to the volume of the cell. It contains abundant cytoplasm, multiple nuclei, and a well-defined sheath which readily permits the use of the injection method for the determination of architecture (Plate I, fig. 1; see also previous work (2)).

The ventricular myocardial conduction system in the dog heart. The myocardial conduction network in the dog was examined in three hearts. In terms of the general criteria discussed above, the histologic differentiation of the myocardial Purkinje fibre in this animal is less marked than that of the other species studied. Its minute anatomy approaches that of ordinary myocardium. If staining is carefully controlled, however, distinct contrasts can be obtained. The Purkinje fibre is wider than a muscle fibre, but rarely more than two or three times the diameter of the latter (Plate II, figs. 1 and 2). The nuclei are usually spherical, although less frequently multiple than in the other species investigated. The perinuclear space is a less pronounced finding, this being probably correlated with the fact that in the dog the Purkinje fibre is more compact and has a greater concentration of intracellular substance. The sheath, however, is characteristic and always present. As a rule, it is extremely delicate and closely applied to the enclosed fibres.

By means of serial sections, the continuity of the myocardial network with the readily identified subendocardial plexus can be definitely demonstrated. As the myocardial trunks branch from the subendocardial network they appear to contain fewer Purkinje fibres than analogous bundles of the other species. Moreover, they tend to divide and course as single or double fibres throughout the myocardium. Consequently, the myocardial Purkinje plexus of the dog is much more diffuse and less readily distinguished from contiguous muscle. Similarly, the exceedingly thin sheath (which is often destroyed as a result of manipulative procedures unless special precautions are taken) renders difficult the casual recognition of the myocardial Purkinje fibre in the dog's ventricle.

Since the histologic studies were not supplemented by reconstructions, our conception of the gross architectural relationship of the myocardial Purkinje network in the dog must necessarily be incomplete. However, from the information obtained by a study of tissues taken from various regions of the

heart, it would appear that the apical half of the septum contains many more myocardial Purkinje fibres than the basal half, and that the bases of the papillary muscles of both ventricles are particularly well supplied. Also, as in the other species, the myocardial branches, as they leave the subendocardial plexus, tend to pass perpendicularly to the endocardium in the left ventricle



Text-fig. 1. Schematised section of heart through both auricles and ventricles, showing endocardial surfaces of the chambers. The Purkinje network is also demonstrated. 1, right auricular cavity. 2, left auricular cavity. 3, aorta. 4, pars membranacea of septum. 5, moderator band in right ventricle containing main right branch. 6, outer wall of right ventricle. 7, subendocardial Purkinje fibres of right ventricle. 8, cut surface of anterior papillary muscle of right ventricle. 9, interventricular septum. 10, subendocardial Purkinje fibres at base of posterior papillary muscle in left ventricle. 11, apex of posterior papillary muscle devoid of subendocardial Purkinje fibres. 12, outer wall of left ventricle. 13, subendocardial Purkinje fibres in left ventricle. A, generally accepted conception of the ventricular conduction system —subendocardial only. B, conception of the ventricular conduction system based on the evidence presented in the present report—Purkinje fibres present also in the muscle substance of the ventricles. 14, myocardial Purkinje fibres in outer wall of right ventricle. 15, Purkinje fibres in substance of anterior papillary muscle. 16, Purkinje fibres in lower two-thirds of interventricular septum. 17, no fibres in upper one-third. 18, myocardial Purkinje fibres in outer wall of right ventricle. (Adapted from Spalteholz.)

and obliquely in the right ventricle. Individual fibres, however, run without regard to the direction of the muscle bundles. For example, Purkinje fibres were often found coursing obliquely between muscle bundles seen in longitudinal and cross-section (Plate II, fig. 1). The interventricular septum is traversed

by myocardial Purkinje fibres which arise from the subendocardial Purkinje network lining the right and left ventricular septal surfaces.

The ventricular myocardial conduction network in the pig heart. The myocardial Purkinje network in the pig was examined in a series of ten hearts. The fibres are readily distinguishable from the surrounding muscle by their staining reaction, great width, rich cytoplasm, sparse fibrillar structure and ever-present sheath (Plate III, fig. 2 and Plate IV, fig. 1). They can be followed from their origin in the subendocardial plexus through their ramification in the outer ventricular walls and interventricular septum. The fibres can be traced to their apparent termination, in many sites, near the epicardial surface of the heart. Here again, because reconstructions were not made, the present description of the gross architecture is incomplete. However, it was noted that the fibres form a network comparable to that in the other species studied. This network is present to a greater degree at the base of the papillary muscles, in the vicinity of the insertion of the auriculo-ventricular valves, and in the apical half of the interventricular septum.

The ventricular myocardial conduction system in the sheep heart. The gross architecture of the myocardial Purkinje network in the sheep, investigated in a series of twenty-two hearts by the injection method, was found to be on the whole similar to that of the ox. The myocardial fibres, continuations of the subendocardial conduction system, ramify throughout the ventricular muscle substance, uniting and branching, to extend in many places as far as the epicardial surface of the heart (Plate IV, fig. 2 and Plate V, fig. 1).

As in the case of the ox, in the outer wall of the left ventricle, the trunks of myocardial Purkinje fibres course for the most part in a direction perpendicular to the subendocardial network (Plate IV, fig. 2). These fibres are especially abundant near the insertion of the papillary muscles and at the base of the ventricle below the mitral cusps. In the outer wall of the right ventricle the arrangement is again similar to that in the corresponding ventricle of the ox. The myocardial Purkinje fibres penetrate the muscle substance as far as the epicardial surface, for the most part forming plexuses which extend obliquely or roughly parallel to the subendocardial Purkinje network (Plate II, fig. 1). In the interventricular septum of the sheep heart, Purkinje fibres are found arising from the subendocardial network of both right and left septal surfaces to penetrate the septal myocardium for varying distances. These fibres are most numerous in the apical two-thirds of the septum, few being found above this level.

The myocardial Purkinje fibre in the sheep heart is identical with that of the subendocardium, the latter having been adequately described by previous investigators (4, 13). Suffice it to say that its degree of differentiation from the ordinary muscle fibre stands between that of the ox and the dog, but closer to the ox (Plate V, fig. 1 and Plate VI, figs. 1, 2).[1]

[1] In view of this it is curious that many of the standard text-books devoted to human histology figure the Purkinje fibre of the sheep as typical for Purkinje tissue. A consideration of the fibres of

DISCUSSION

Of the early workers, Tawara (13) stands out as one of few who made any attempt to describe the anatomy of the myocardial Purkinje network deep in the substance of the mammalian ventricular wall. In his monograph on the conduction system he stated that in the sheep heart histologic studies revealed the presence of Purkinje fibres not only directly beneath the endocardium but also very often lying in the connective tissue septa between the heart muscle bundles. Although he made a number of preparations of the entire thickness of the ventricular wall he could not determine from a study of these whether or not the myocardial Purkinje fibres reached the epicardium. In the calf's heart also, the above author found similar myocardial fibres. Again, although he made a special search for fibres lying between the muscle bundles of the outer ventricular walls in the dog, he stated that he had not with certainty demonstrated such structures. Nor was he able to find Purkinje fibres in the muscle substance of the interventricular septum, thus concluding that there was little likelihood of a connection in the septum existing between the Purkinje arborisation of the left and right ventricles other than by way of the main branches of the Bundle of His.[1]

On the other hand, Whalin (14) described the presence of a myocardial Purkinje plexus in the interventricular septum of a single calf's heart. This plexus was a continuation of the subendocardial Purkinje network on the septal surfaces of the right and left ventricles. Cardwell and Abramson (2), in an examination of more than fifty ox hearts, found that the myocardial Purkinje system was consistently present not only in the interventricular septum but also throughout the muscle substance of the outer walls of both ventricles, in many places extending as far as the epicardial surface of the heart. According to them, the fibres in the septum extended through its entire thickness, connecting the subendocardial Purkinje plexus of one ventricle with that of the other. The above investigators concluded that the fibres of the myocardial Purkinje network in the ox heart collectively are comparable in number and extent of distribution with those of the subendocardial reticulum. Later Whalin (15) presented further evidence for the presence of a transeptal myocardial Purkinje network in a series of calves' hearts.

Lewis and Rothschild (9), in their investigation on the spread of the impulse in the ventricles of the dog, although aware of what they believed to be the limited presence of myocardial Purkinje fibres, did not consider them in

various species will show that the sheep is analogous rather than representative, a fact that was recognised by Tawara (13), de Witt (4), and Kulbs (6). Some workers considered the species to be so marked that they evolved a hypothesis in which it was proposed that the degree of differentiation was correlated with the zoological order; for example, the Ungulates such as the ox, sheep, swine, antelope, horse, and camel possess a highly specialised Purkinje structure as opposed to man, dog, cat, rabbit, and rat, which have a much less differentiated one, more nearly resembling ordinary muscle.

[1] For further reference to the early literature on the myocardial Purkinje network, see previous work (Cardwell and Abramson (2)).

the interpretation of their results. These investigators stated that the excitation wave is distributed throughout the subendocardial Purkinje system and then through the ventricular musculature by direct penetration in a radiating fashion from the network. Further, according to Lewis (8), the wave is carried from one muscle band to the overlying one, travelling in a plane which as a rule is at right angles to the direction of the fibre, i.e. the excitation is propagated without regard to the anatomic direction and continuity of the muscle bundles. According to Dale and Drury (3) also, the impulse spreads rapidly and practically simultaneously through the Purkinje tissue lining the chambers and has only to travel through a "small thickness of muscle" to reach the epicardial surface. On the other hand, Rothberger (12), on the basis of recent work in the calf and ox hearts (2, 14), assumed the presence of similar transeptal Purkinje fibres in the dog's ventricles in order to help explain some of his experimental results. Wilson and his associates (16), although not denying that such fibres may exist, do not attach much significance to them. Contrary to all these views is that of Robb (11), who considers the impulse to be propagated through the muscle substance of the ventricles along the various muscle bundles making up the walls, an hypothesis originally held by Gotch (5).

It would appear from the above, therefore, that hitherto (with the exception of the work of Rothberger) the physiology of the ventricular conduction system is premised on the erroneous anatomic concept that the Purkinje fibres are subendocardial only (text-fig. 1 A). The investigation reported in this paper reveals that a myocardial Purkinje network similar to that in the calf and ox hearts, is also present in the dog, pig and sheep (text-fig. 1 B). In both staining properties and structure this network is essentially like the subendocardial system of which it is a continuation. The finding of a complex myocardial Purkinje network in the outer ventricular walls and in the interventricular septum of the dog seems to us to be of special significance, since this animal has been the subject of most of the definitive and critical physiologic experiments on the conduction system.

SUMMARY

1. Evidence is presented to show that the anatomic concept of the ventricular conduction system, hitherto held, is incomplete. The Purkinje arborisation of the ventricles which is generally believed to be only subendocardial in location, is demonstrated to ramify throughout the outer ventricular walls and in the interventricular septum.

2. By means of injection and histologic technique this myocardial Purkinje network is found to be directly continuous with and structurally identical with that of the subendocardium.

3. Criteria for the recognition of these myocardial Purkinje fibres in the ox, sheep, pig and dog hearts are defined.

4. In view of the data presented in this report, attention is called to the necessity of considering the myocardial Purkinje network in the evaluation of conduction phenomena in the ventricles.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Ox heart. Portion of cut surface of left ventricular wall, showing injected Purkinje fibres. A, subendocardial Purkinje fibres at the base of the anterior papillary muscle seen through the endocardium. B, myocardial Purkinje network, continuation of the subendocardial plexus, extending grossly perpendicular to the endocardium. C, subepicardial fat.

Fig. 2. Dog heart. Subendocardial Purkinje fibre. A, endocardium. B, Purkinje fibre. C, myocardium. ×337.

Plate II

Fig. 1. Dog heart. Myocardial Purkinje fibre in outer wall of left ventricle. The fact that myocardial conduction fibres do not necessarily parallel heart fibres is well shown. A, longitudinal section of heart muscle. B, vein. C, sheath. D, longitudinal section of myocardial Purkinje fibre. E, cross-section of heart muscle. ×337.

Fig. 2. Dog heart. Myocardial Purkinje fibres surrounding small arteriole in interventricular septum. A, lumen of arteriole. B, Purkinje fibre. C, heart muscle. D, sheath. E, fat cells. ×225.

Plate III

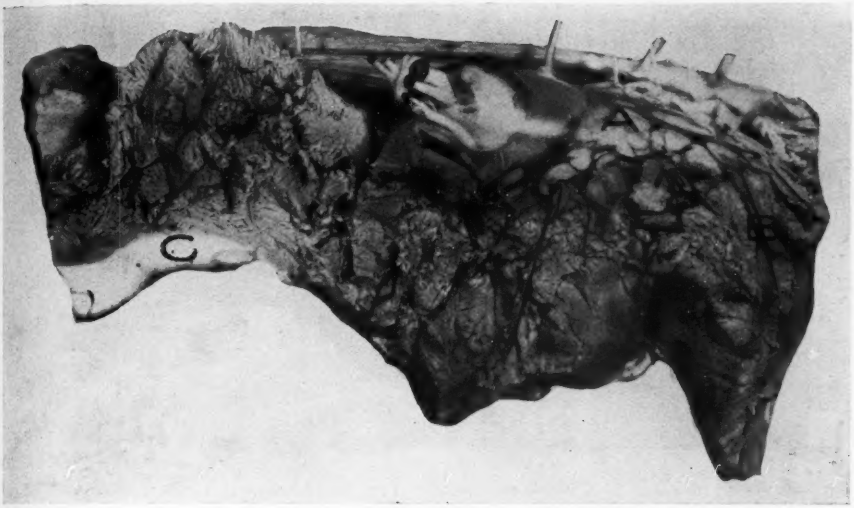
Fig. 1. Pig heart. Subendocardial Purkinje fibres. A, endocardium. B, sheath. C, Purkinje fibres. D, fat cells. E, heart muscle. ×225.

Fig. 2. Pig heart. Myocardial Purkinje fibres in outer wall of left ventricle. A, myocardium. B, myocardial Purkinje fibres. C, sheath. The morphological difference between Purkinje fibre and heart muscle is striking. ×525.

Plate IV

Fig. 1. Pig heart. Myocardial Purkinje fibre in interventricular septum. A, myocardium. B, cross-section of Purkinje fibres. C, sheath. The peripheral distribution of the fibrils gives the cell membrane of the Purkinje fibre a striated appearance. ×450.

Fig. 2. Sheep heart. Portion of cut surface of left ventricular wall. The Purkinje fibres have been injected with India ink. A, subendocardial Purkinje fibres seen through the endocardium. B, anterior papillary muscle. C, myocardial Purkinje fibres, continuations of the subendocardial network, extending through the muscle substance of the outer wall in the direction of the epicardium. D, epicardium.



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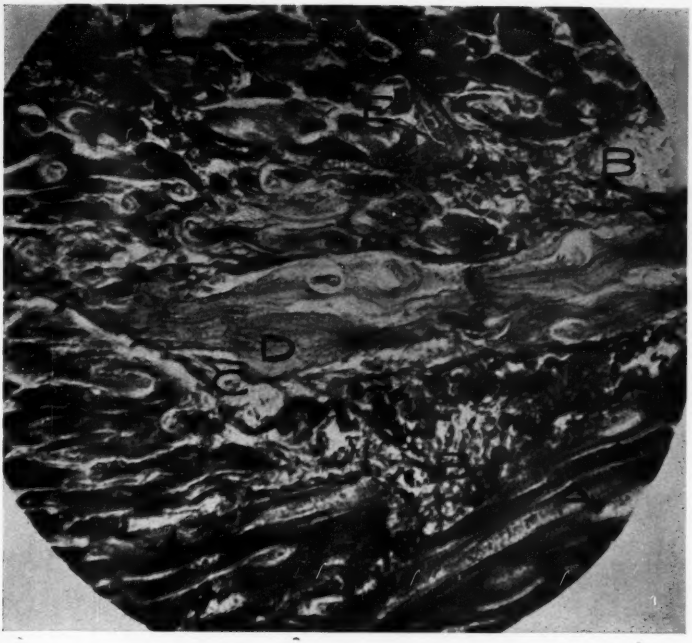

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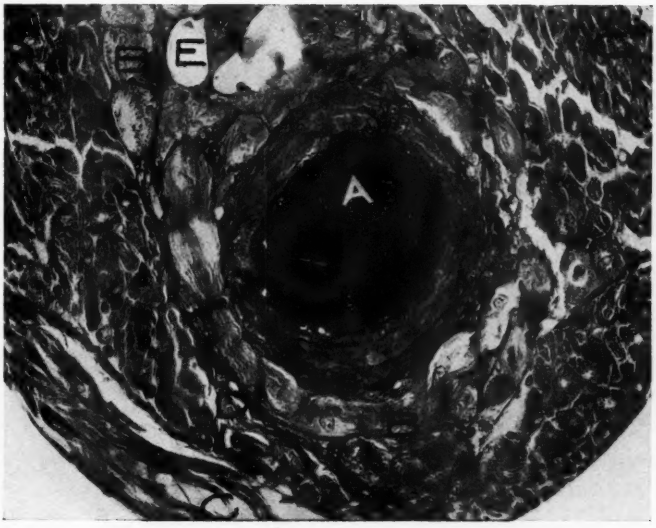

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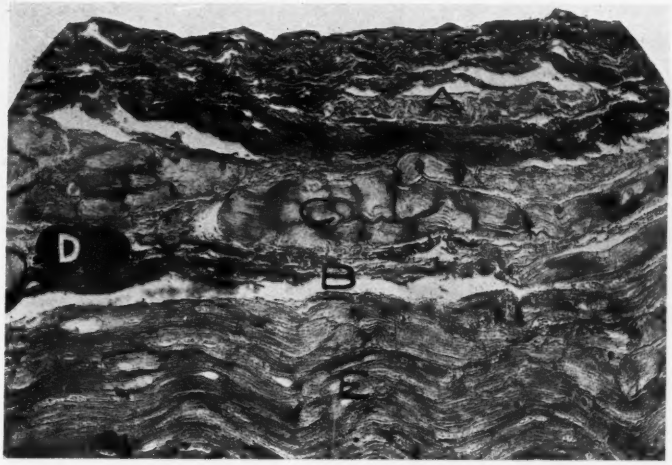

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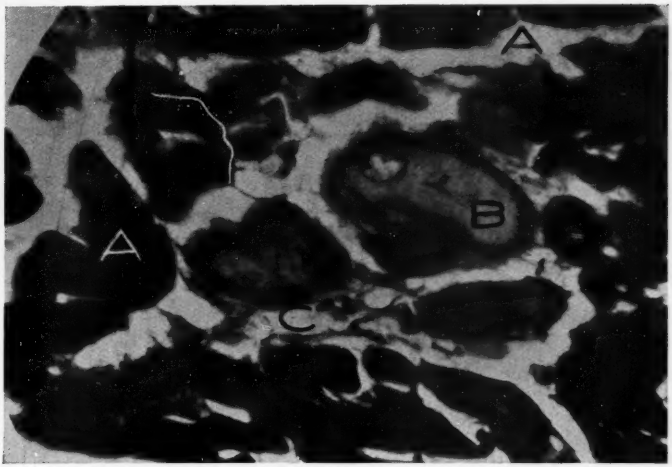

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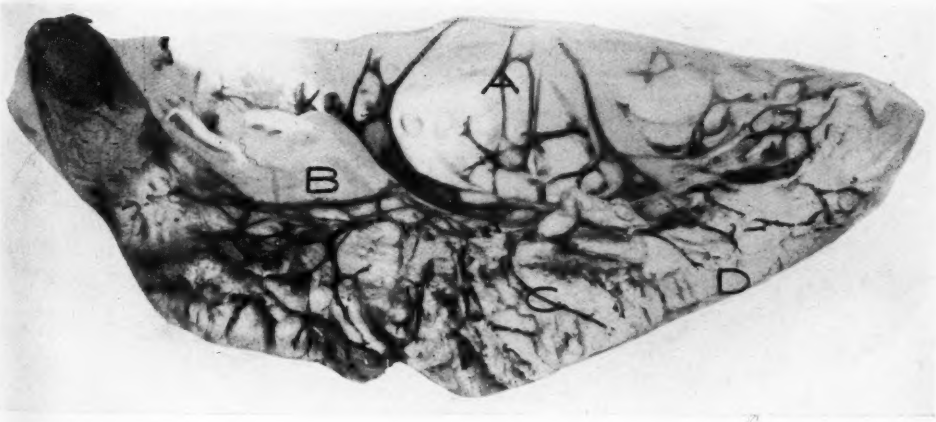

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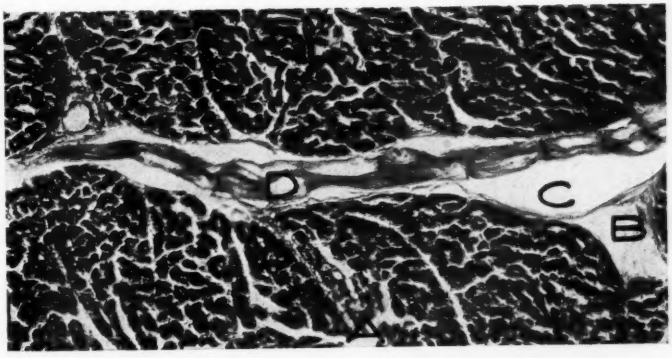

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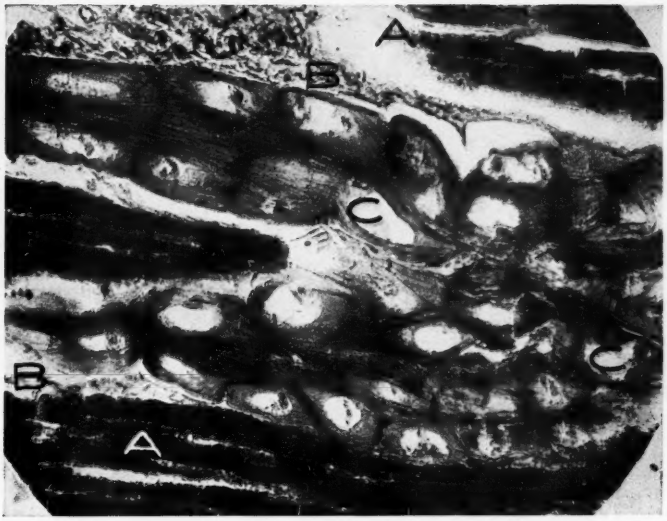

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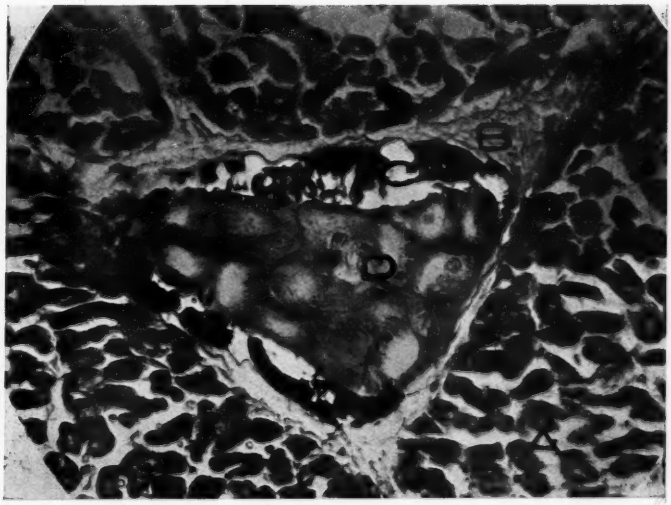

Fig. 2

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PLATE V

Fig. 1. Sheep heart. Portion of anterior wall of right ventricle, showing injected Purkinje fibres. In one part the endocardium and part of the muscle have been dissected away, in order to show the myocardial Purkinje fibres. A, subendocardial Purkinje fibres seen through the endocardium. B, anterior papillary muscle. C, myocardial Purkinje network in the outer wall of the right ventricle.

Fig. 2. Sheep heart. Myocardial Purkinje fibre in interventricular septum. A, cross-section of heart muscle. B, sheath of Purkinje fibre. C, perifibrillar space. D, longitudinal section of Purkinje fibre. Note lack of parallelism in direction between Purkinje fibre and heart muscle. ×225.

PLATE VI

Fig. 1. Sheep heart. Myocardial Purkinje fibres dividing into two parts in outer wall of left ventricle. A, myocardium. B, sheath. C, Purkinje fibres. Note syncytial nature, multiple nuclei and perinuclear space of Purkinje fibres. ×337.

Fig. 2. Sheep heart. Purkinje fibre in the myocardium of the interventricular septum. The subendocardial Purkinje network had previously been injected with India ink. A, myocardium. B, sheath. C, Injection mass lying in perifibrillar space between sheath and enclosed fibres. D, Purkinje fibres. The fact that the India ink appears in the sheath surrounding a fibre deep in the interventricular septum demonstrates the continuity between the subendocardial and myocardial Purkinje fibres. ×337.

THE DEVELOPMENT OF TOOTH GERMS *IN VITRO*

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INTRODUCTION

Much experimental work has been done recently on the growth of isolated embryonic tissues *in vitro* with a view to studying the capacity of these tissues for self-differentiation. Although deprived of a vascular and nervous system and connections with neighbouring structures many tissues develop in a comparatively normal way during cultivation. Bone, for example, differentiates readily *in vitro* (Fell, 1932), and since bone and dentine are very closely related it seemed possible that dentine might develop under the same conditions. If this were so a new field of dental research would be opened, as dental tissue cultures would provide ideal material for analysing many of the physiological conditions of tooth development. The experiments recorded in the present communication were therefore undertaken in order to find whether embryonic dental tissues would differentiate normally when isolated and cultivated *in vitro*.

Probably the first experiments on the explantation of teeth on record were those of Cadmus, who, it may be remembered, explanted in the soil dragons' teeth which grew into armed men (Stesichorus, 6 B.C.). Apart from these legendary experiments of Cadmus which had such singular results, very little work has been done on the self-differentiating capacity of dental tissues outside the mouth.

Legros and Magitot (1874) removed tooth germs from dogs 21–28 hours after birth and grafted them subcutaneously into guinea-pigs or adult dogs. The grafts were portions of maxilla including tooth germs, isolated enamel organ, complete tooth germs, pulp and the isolated dentine cap. All tooth germs grafted into guinea-pigs were lost by suppuration or resorption. Of the grafts in dogs, seven out of the twenty-six complete tooth germs formed enamel and dentine, and in the isolated pulp grafts, two out of sixteen formed dentine. A few of the grafts showed no growth at all and the majority of them were lost by resorption or suppuration. As these experiments were done in the days before asepsis was practised the results obtained were highly commendable.

Further work on the transplantation of tooth germs was done by Huggins, McCarroll and Dahlberg (1934). They grafted the unerupted canine teeth of dogs between the ages of 3–6 weeks on to the connective tissue of the abdominal wall or thigh of the same dogs. They removed the calcified cap of dentine and enamel and explanted the enamel epithelium and the dentine papilla separately.

The transplants of the isolated epithelial layer formed no new enamel, but the isolated dentine papilla had produced dentine 14 days after transplantation. They also grafted the enamel epithelium and dentine papilla together in various ways. Both enamel and dentine differentiated in this series, enamel being deposited only on dentine, but the dentine being formed independently of the enamel. In other experiments the centre of the pulp was completely freed of its odontoblasts and transplanted; no dentine was formed even after 29 days, although the odontoblast layer which was removed from the pulp and transplanted separately formed small islands of dentine during the same period. The results of Huggins, McCarroll and Dahlberg thus showed that the soft tissues of tooth germs were able to differentiate readily when transplanted into abnormal surroundings outside the mouth.

As far as the present writer is aware no previous work has been done on the growth of teeth *in vitro*.

MATERIAL AND METHODS

The tooth germs of 18–21-day rat embryos were cultivated. Molars and incisors which had not yet begun to form dentine were explanted whole; in those incisors where a cap of dentine had formed on the tip, this cap together with the enamel epithelium was removed and the dentine papilla itself was explanted. In performing this last operation some of the odontoblasts remained adherent to the cap of dentine whilst some were left behind on the tip of the dentine papilla. In some of the explants a little of the enamel epithelium also remained attached. Owing to the small size of the dentine papilla it was not possible to remove the ameloblasts completely without irreparable damage to the tissue.

As explantation had to be done with the strictest aseptic precautions and the removal of teeth from post-foetal rats under such conditions is almost impossible, the necessary material had to be obtained from embryos.

The tooth germs for cultivation were dissected out with a cataract knife and a needle from both upper and lower jaws and washed in saline. In order to determine the stage of development of each tooth, one tooth was removed for explantation, whilst the corresponding tooth from the opposite side of the jaw was fixed, either *in situ* in a fragment of jaw or after removal, and sectioned to serve as a control.

The culture methods employed were the hanging-drop and watch-glass techniques. In the hanging-drop method the explants were placed in a clot of medium composed of one drop of fowl plasma and one drop of embryo extract made with Pannett and Compton's saline from 10–11-day chick embryos. The cultures were grown on $1\frac{1}{4}$ in. square cover-slips inverted over a hollow-ground slide sealed with paraffin wax and incubated at 37° C. The explants were transferred to fresh medium every 72 hours. In the watch-glass method four drops of fowl plasma and four drops of embryo extract were mixed in a watch-glass which was enclosed in a Petri dish surrounded by cotton-wool saturated

in sterile water. A large hole was cut in the cotton-wool to allow trans-illumination of the explants for microscopic observation. The tissue which was grown on the surface of the clot was removed from the old medium every 72 hours, washed in saline and transferred to a fresh clot.

The explants were fixed at different periods of growth in Zenker's solution containing 3 per cent. glacial acetic acid for 30 min., then washed, dehydrated, cleared in cedar-wood oil and embedded in paraffin wax. Serial sections were cut and were stained with iron haematoxylin and chromotrope.

THE STRUCTURE OF THE TOOTH GERMS AT THE TIME OF EXPLANTATION

As mentioned above the stage of development at the moment of explantation was ascertained from the series of controls obtained by fixing and sectioning one of each pair of teeth and cultivating the corresponding tooth.

Whole tooth germs

The whole tooth germs at the time of explantation consisted of the dentine papilla, which was a mass of stellate connective tissue cells, surrounded by the enamel organ. In some of the explants the peripheral cells of the dentine papilla were in an early stage of differentiation into odontoblasts; they were rounded in form with large nuclei and a small amount of cytoplasm. In other explants the formation of odontoblasts had not yet begun. In the molars the shape of the cusps was already determined by the enamel organ dipping into the dentine papilla.

The dentine papilla

The dentine papilla of the incisors (Plate I, fig. 1), which was removed from the teeth of 19–21-day embryos, consisted of connective tissue cells with some odontoblasts at the periphery, mostly near the tip. As mentioned above a number of odontoblasts were torn away in the process of removing the dentine cap. The connective tissue cells towards the base had not yet begun to differentiate into odontoblasts. In some of the explants a small part of the internal enamel epithelium was present in the basal region, owing to the difficulty of removing it without serious injury to the papilla itself.

GROWTH AND DIFFERENTIATION OF THE EXPLANTS

Observations on the living explants

The explants did not enlarge during cultivation but usually contracted slightly, particularly those of the dentine papilla. In the hanging-drop cultures the original odontoblasts and ameloblasts were clearly seen as bands of columnar cells. During cultivation odontoblasts and dentine continued to differentiate. The newly formed tissues were not seen quite so clearly but were usually marked by a translucent area. In some of the explants of the dentine papilla the tip, with its peripheral layer of odontoblasts, contracted and degenerated

by the third day. This was due to the fact that this portion of the papilla was rather narrow, and in removing the dentine cap it was severely injured and unable to recover *in vitro*. In the watch-glass cultures the original ameloblasts and odontoblasts could be distinguished as light bands. Considerable growth in the cusps of the molars was observed during the period of cultivation, and in the incisors, in some of the cultures, some distortion was noticed. For detailed observation of living tissue the watch-glass method is not very suitable.

Histological differentiation

Histological examination of the explants at different stages of cultivation showed normal differentiation of the cells of the dentine papilla to form odontoblasts and dentine, although a few explants degenerated at about the third or fourth day. The formation of the dentine proceeded much more slowly *in vitro* than *in vivo*. An explant after 20 days *in vitro* (Plate I, fig. 2) showed no more dentine than a normal tooth germ 4 days after the formation of odontoblasts.

Dentine papilla

In the absence of any epithelium, the odontoblast layer invaginated into the pulp and deposited irregular bands of dentine (Plate I, fig. 3). By the third day, wherever a fragment of enamel epithelium existed, a new odontoblast layer had been formed adjacent to it. The enamel epithelium continued to grow and showed numerous mitoses. Its extension was always followed by the development of an underlying layer of odontoblasts (Plate II, fig. 4).

The various stages in the development of these new areas of odontoblasts could be followed in cultures of different ages. After 2 days' growth *in vitro*, at the future site of the odontoblast layer adjacent to the ameloblasts, the connective tissue cells had begun to change their appearance, becoming larger, rounded and containing relatively larger nuclei. By the third day these cells had enlarged still further, were very closely packed, and changed from a rounded to a cylindrical form. The oval nuclei lay at the ends of the cells farthest from the site of the future dentine. The dental surface of the cytoplasm was flat but continued into protoplasmic strands, passing into a layer of collagenous substance which had now been deposited in the odontogenic zone. By the fourth day a band of normal tubular dentine had been formed, and the protoplasmic strands were enclosed in the tubules as dentinal fibrils (Plate II, figs. 5, 6).

After the fourth day the formation of dentine progressed very slowly until the fourteenth day, when the odontoblasts began to degenerate. This degeneration of odontoblasts continued, and on the twentieth day the connective tissue cells also began to degenerate rapidly. As described above, in some of the cultures the odontoblasts and the connective tissue cells at the tip degenerated soon after explantation. This necrotic tip was removed, leaving part of the dentine papilla composed of connective tissue cells alone, which remained alive but failed to differentiate. If in these cultures some ameloblasts were

present, new odontoblasts and dentine were formed irrespective of the fact that the tip had been removed.

Whole tooth germs

The histological differentiation in the whole tooth germs was the same as in the cultures of the dentine papilla. The peripheral pulp cells which were in an early stage of differentiation formed odontoblasts and dentine. Those cultures where the peripheral pulp cells at the time of explantation had not yet begun to differentiate did the same eventually. The presence of the enamel organ controlled the position of the newly formed dentine which appeared next to the ameloblast layer. As the enamel organ had already determined the shape of the cusps by dipping into the dentine papilla, regular cusps were formed by the new dentine, except that in some of the explants there was some distortion of the tooth as a whole.

The development of abnormal dentine

In some of the cultures, besides the normal tubular dentine, an abnormal dentine was formed comparable to the "scar tissue" described by Fish (1932). In the early stages of the development of this "scar tissue", some of the odontoblasts became rounded, and instead of forming tubular dentine produced a mass of collagenous fibrillar material which later became much more abundant. The odontoblast layer disappeared, but irregular-shaped cells with large nuclei were found embedded in this "scar tissue" and possibly represented abnormal odontoblasts (Plate II, fig. 7).

DISCUSSION

The results obtained from these experiments confirm the conclusions of Huggins, McCarroll and Dahlberg that isolated dental tissue is self-differentiating. Whereas their evidence was derived from the transplantation of tooth germs into the abdomen, the results of the present study were obtained from the cultivation of tooth germs *in vitro*, which, by eliminating all the vascular and nervous connections, produced a more complete isolation than grafting.

As described above, in the absence of the enamel epithelium, the peripheral layer of odontoblasts produced convoluted bands of dentine, but wherever dentine was formed in contact with the epithelium it was deposited as regular sheets. This result clearly showed that one of the functions of the enamel epithelium is to determine the morphological structure of the tooth, thus confirming the views of earlier investigators (von Brunn, 1887, 1891; Ballowitz, 1892).

Of particular interest is the question of the influence of the enamel epithelium over dentine development. It had previously been held that this epithelium, which even occurs in teeth which do not form enamel, is necessary for the deposition of dentine. Hertwig (1874) described the presence of an epithelial sheath in certain Amphibia, which he believed was essential for tooth develop-

ment. Tomes (1876) discovered the existence of an enamel organ in the armadillo, whose teeth have no enamel, and later found that in all Edentata an enamel organ occurs although enamel is absent. Von Brunn (1887, 1891) carried the investigations further and showed that many Mammals in other groups possess enamel epithelium in the absence of enamel, notably the Rodents, whose incisors have no enamel on the dorsal surface but yet are entirely invested by an enamel organ. He came to the conclusion that the epithelial sheath is responsible for the development of odontoblasts and dentine, and that its primary function is that of determining the form of the future tooth. Ballowitz (1892), in his paper on the enamel organ of the Edentata, confirmed von Brunn's conclusions.

Since Huggins, McCarroll and Dahlberg obtained the formation of dentine in the isolated dentine papilla in the absence of the epithelial layer, their results were not in entire agreement with the views of the previous authors mentioned above. They therefore modified von Brunn's statement that "where there is no epithelial sheath, there can be no odontoblast formation and consequently no dentine", and concluded that odontoblasts that have arisen as a result of epithelial influence on connective tissue do not further need for their function the presence of an epithelium.

The results of the present study fully confirm the conclusions of Huggins, McCarroll and Dahlberg. The writer's experiments have shown that odontoblasts differentiated *in vitro* only when ameloblasts were present. Moreover, if the original odontoblast layer degenerated in an explant containing no epithelium, there was no further differentiation of the pulp; the connective tissue cells survived as such, which suggested that they cannot develop into odontoblasts in the absence of the enamel epithelium. It is therefore probable that the enamel epithelium, though not necessarily the whole of the enamel organ, has an organising effect on the dentine papilla inducing odontoblast formation. Once the connective tissue cells have reacted to this influence and have differentiated into odontoblasts, the presence of the enamel epithelium is not necessary for the deposition of dentine.

The "scar tissue" produced in the cultures is interesting, as it is similar to the early stages of "scar tissue" found in the teeth of guinea-pigs suffering from scurvy (Fish and Harris, 1934). The nature and significance of this abnormal dentine is being investigated further.

SUMMARY

1. Whole or partial tooth germs cultivated *in vitro* showed remarkable powers of histological differentiation. The dentine papilla developed normally, forming odontoblasts which deposited normal tubular dentine.

2. The presence of the internal enamel epithelium is essential for odontoblast formation.

3. Dentine formation could take place in the absence of the enamel epithelium provided that odontoblasts were present in the explant.

4. One of the functions of the enamel organ is to determine the gross morphological structure of the tooth.

5. "Scar tissue", similar to the "scar tissue" found in the teeth of animals suffering from scurvy, occurred in some of the cultures.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Longitudinal section of the dentine papilla after removal of the dentine cap, showing the general histological structure (× 57).

Fig. 2. Longitudinal section of a 20-day culture of the dentine papilla, showing the convoluted bands of dentine formed in the absence of the enamel epithelium. Also the "scar tissue" formed in the same culture (× 110).

Fig. 3. Higher magnification of the convoluted dentine shown in Fig. 2. The original odontoblasts have almost completely degenerated (× 460).

PLATE II

Fig. 4. Longitudinal section of an 8-day culture of the dentine papilla showing the new layer of odontoblasts and dentine formed adjacent to the ameloblast layer (× 360).

Fig. 5. Transverse section of an 11-day culture of a molar showing the dentinal tubules (× 550).

Fig. 6. Longitudinal section of a 12-day culture of an incisor showing the sheet of tubular dentine formed when the enamel epithelium was present (the latter was removed after cultivation) (× 450).

Fig. 7. Higher magnification of "scar tissue" shown in Fig. 2, showing its fibrillar structure and the irregular-shaped cells embedded in it (× 380).

ABBREVIATIONS

a.	ameloblasts	*e.c.*	embedded cells
c.d.	convoluted dentine	*f.*	fibrils
d.	dentine	*o.*	odontoblasts
d.o.	degenerate odontoblasts	*s.t.*	"scar tissue"
d.t.	dentine tubules		

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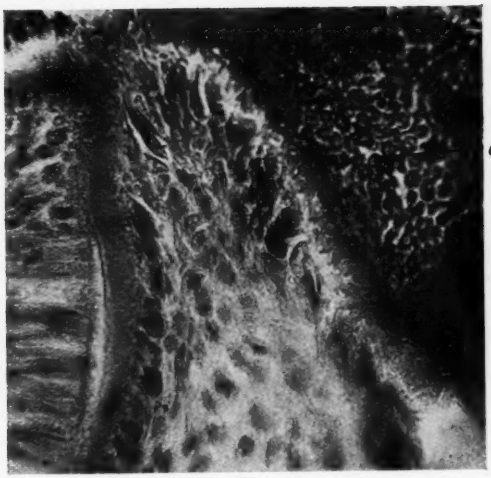

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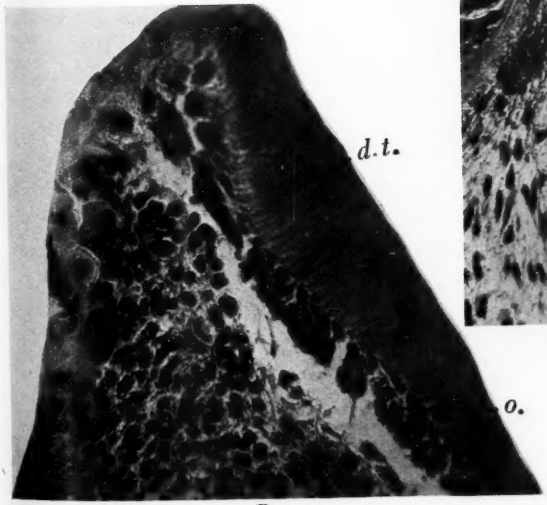

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ON THE STAINING OF LIPOID GRANULES IN LEUCOCYTES[1]

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THERE are relatively few investigations reported in the literature on the subject of the lipoid granules in leucocytes and even these contain many contradictory statements. No simple, but at the same time reliable, technique for the staining of lipoids in leucocytes has been evolved. Reinvestigation of this subject is important in view of the importance of fat and lipoid metabolism in the explanation of physiological and pathological processes and because of the role played by the leucocytes in pathological and immunological reactions.

Two views are held at present concerning the significance of lipoid granules in leucocytes. According to the first, based on the original or modified Cesaris-Demel technique (1909), lipoid granules are very few in number and are contained only in a few cells; these granules are thought to be of phagocytic origin or due to the degeneration of the cytoplasm (Cesaris-Demel, 1909; Carletti, 1908; Comesatti, 1907; Carminati, 1930; Martelli, 1909; Romanelli, 1907). According to the second view, lipoid granules are numerous and a constant physiological constituent of the leucocytes (Savini, 1921; Sehrt, 1927; Goldmann, 1929). Intermediate views are also expressed; for instance, Jousset and Troisier (1907) find only a small number of lipoid granules in leucocytes and suggest that these are physiological constituents, while Goreczki and Slonimski (1924) have differentiated between physiological and pathological lipoid content of leucocytes.

In the early stages of the present work the techniques described in the literature were repeated in order to determine the most simple and reliable method for staining lipoid granules in leucocytes. It was concluded that the technique of Cesaris-Demel, in both its original and modified forms, is not suitable for staining lipoid granules in leucocytes, and therefore the data of this author and his school are not acceptable. In this connection it is interesting to note that Hammar (1912) found that "brilliant-cresylblau" stains certain degenerated cell constituents a pink colour (metachromatic staining), and, as this dye is a part of the staining solution used by Cesaris-Demel, the latter author has erroneously described these degenerated granules as sudanophil (lipoid) in nature.

[1] This paper is a translation and extension of a short note in Hungarian entitled "On the lipoid content of the leucocytes (fat-staining method for leucocytes)", published in a limited edition in *Publications of the Department of Histology and Embryology of the University of Szeged*, No. 7, 1934.

It was found impossible to stain lipoid granules in leucocytes with Savini's technique.

Sehrt's technique gave constant positive results but has the disadvantage of being very long and complicated and therefore unsuitable for the examination of numerous specimens.

Goldmann claims to have obtained practically the same results as Sehrt, but his technique (published in Russian) was not available to us.

Kaufmann and Lehmann (1926) observed that histochemical fat-differentiating staining reactions are not specific as was previously thought, and found that Sudan III is the most sensitive stain for all kinds of lipoids. For this reason the present author set out to evolve a technique for staining lipoid granules in leucocytes using Sudan III.

Formerly Sudan III was used dissolved in 70–96 per cent. alcohol, or in acetone-alcohol (Herxheimer), but Kaufmann and Lehmann, in their later work (1929), noted that these high percentage alcoholic solutions dissolve a great deal of the cell lipoids. This was corroborated by Romeis (1929), who recommended the use of a specially prepared solution of Sudan III in 40 per cent. alcohol, which does not dissolve the cell lipoids and stains the finest granules a bright red colour.

The present writer observed that immersion of blood films in the solution of Romeis for 24 hours at room temperature failed to give a positive reaction, but on staining films in the incubator at 36° C. for 2 hours it was found that the finest lipoid granules in the leucocytes were stained a bright red colour. This difference we believe to be due to the fact that at 36° C. the lipoids are liquid and mix with the stain, whereas at room temperature they are solid and the stain does not readily penetrate them. After much experiment it was found that the addition of carbolic acid to the staining solution enabled a positive staining reaction (at 36° C.) to be obtained in 25–30 min. Phenol and naphthol derivatives were found to act in a similar manner, but carbolic acid itself gave the best staining results. The carbolic acid probably acts as a mordant and facilitates the attachment of the Sudan III to the lipoids (lipoid-carbolic acid-Sudan III). It was found that the staining power of the Romeis solution diminished from day to day probably owing to the changing colloidity of the solution. For strict comparison it was therefore necessary to stain the different materials under examination in the same solution at the same time. Froboese and Spröhnle (1928), however, observed that if to an alcoholic solution of Sudan III a small quantity of water is added, with the increased colloidity of the solution the number of precipitating molecules and the staining power are also increased. For this reason, in contrast with Romeis, the present author used the freshly made strongly colloidal Romeis solution (as described below) and found that the staining time was reduced to 10 min. at 36° C. or 5 min. at 56° C.

I. PREPARATION OF THE STAINING SOLUTION

(1) In a litre flask of Jena glass place 1 gm. of finely powdered Sudan III in 200 c.c. of 96 per cent. alcohol. Boil over a water bath for about 5 min. Add 5 per cent. (by volume) of melted carbolic acid. (Place a loose glass stopper in the neck of the flask so that the evaporated alcohol condenses and drops back into the flask.)

(2) Shake thoroughly the boiling solution and filter into a well-stoppered jar, which is placed in the refrigerator for 24 hours. (Precipitation occurs of the parts of the Sudan III with feeblest staining power.)

(3) Filter. Measure the quantity of fluid and add drop by drop with continuous shaking distilled water until the alcoholic concentration is reduced to 80 per cent. (e.g. to 200 c.c. of solution add 40 c.c. distilled water).

(4) Allow the slightly cloudy solution to stand at room temperature for 24 hours. Slight precipitation occurs.

(5) Filter. Keep this dark red transparent solution as stock in a well-stoppered jar.

(6) Prepare staining solution freshly from the above stock solution as required by adding drop by drop with continuous shaking an equal quantity of distilled water. This reduces the alcoholic concentration to 40 per cent. and results in a strongly colloidal opaque solution. Romeis allowed this solution to stand for 24 hours for precipitation of part of the Sudan III and filtered before using. But, for the reason given above, the present writer uses the staining solution as soon as prepared from the stock solution and without filtering.[1]

II. STAINING PROCESS

(1) In spreading the blood film on a carefully cleaned slide, incline the cover-slip at an angle of about 25° to the slide. This ensures a thin blood film and the leucocytes appear larger than in the thicker film which would result from inclining the cover-slip at a bigger angle, and thus cytological changes are easier to recognise.

(2) Dry film in 36° C. incubator or at room temperature for ½–1 hour. (Preparations older than 1 week are not suitable for staining. Preparations should not be allowed to lie for any length of time in a bright light, which oxidises the lipoids.)

(3) Cover film with stain prepared as above described and place in incubator at 56° C. for 5 min.

(4) Wash with 40 per cent. alcohol for 1 min.

[1] The preparation of the staining solution is described in detail because Romeis, on the basis of his fractional precipitation experiments, suggested that commercial Sudan III is not a homogeneous substance. He describes it as containing three components, Sudan red, Sudan orange and Sudan yellow, which are successively precipitated by progressive dilution of the alcoholic solution of Sudan III with water. Romeis claims that Sudan orange has the strongest staining power and the gradual diluting of the 96 per cent. alcoholic solution of Sudan III above described ensures a more concentrated solution of Sudan orange in 40 per cent. alcohol than would otherwise be obtained by merely dissolving Sudan III directly in 40 per cent. alcohol.

(5) Wash thoroughly in running tap water for 1–2 min. to remove the precipitate.

(6) Counterstain, preferably with Weigert's iron haematoxylin, for 30–60 sec.

(7) Rinse with tap water.

(8) Differentiate in acid alcohol (HCl 0·5 c.c., 40 per cent. alcohol 100 c.c.) for 2–3 sec.

(9) Wash in tap water for a few minutes.

(10) Dry slide in air or 36° C. incubator and examine with one-twelfth inch objective in glycerine (cedar-wood oil dissolves Sudan III), using an opal glass bulb which gives white illumination.

Sudan III crystalline deposit rarely occurs, but if present can readily be distinguished from stained lipoid granules. The preparations unfortunately are considerably faded a week after making, but this may be prevented if necessary by keeping the stained films in pure glycerine or by mounting them with Zwemer (1933) glychrogel solution, in which case fading does not occur for several weeks.

It has been noted that commercial specimens of Sudan III vary greatly in staining properties, this applying not only to specimens obtained from different sources but also to those obtained from the same manufacturer. This may account for the discrepancies in the observations of earlier workers in this subject. The colour of the powdered Sudan III (dark red or orange) is no indication of its staining power. The staining process described above can be utilised for determining the staining powers of various specimens of Sudan III; a negative staining result signifies absence of full staining power of the dye.

Briefly, the following observations on the lipoid content of leucocytes were made with the above staining process. Lipoid granules are a constant physiological constituent of the granulocytes. This agrees with the work of Sehrt (1927) and Goldmann (1929). Only some of the monocytes and no lymphocytes contain lipoid granules. In the eosinophils the lipoid granules stain very easily and assume a brighter red colour than in the other leucocytes. This possibly signifies a different chemical nature of these granules. In this connection it may be noted that Berg (quoted by Goreczki and Slonimski, 1924) observed that the lipoid granules of the eosinophils contained tyrosine, which Berg maintains increases the affinity of the granules for Sudan III. The variable reaction of the monocytes to Sudan III is probably evidence of the heterogeneous character of these cells. Like Sehrt, the present author failed to find any evidence of the hypothetical lipoid membrane of the red blood corpuscles. As the figure shows, the appearance and arrangement of the lipoid granules (stained with Sudan III) in the various leucocytes is identical (except for colour) with those of the granules stained by the customary blood staining methods or by the oxydase reaction. The above observations support the modern conception that the granules of the leucocytes are important functional constituents of the cells

and contain lipoids and albumins and, further, enzymes concerned in the respiratory activity of the cells.

It may be observed that the number and size of the lipoid granules in leucocytes do not alter after several days' fasting, or after a large meal. The latter finding is contrary to the view concerning the role of leucocytes in fat transport, the leucocytes being said to carry fat granules.

It is interesting to note that the young, recently formed white blood cells (observed, for instance, in myeloid leukaemia) are densely laden with lipoid

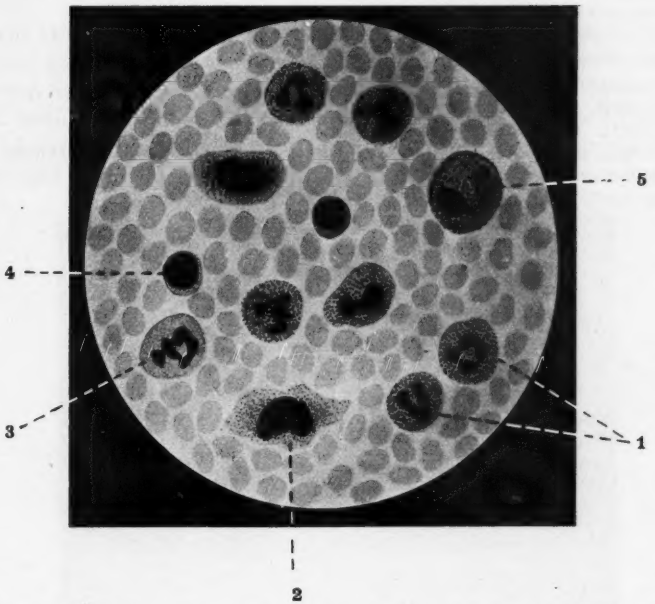

Fig. 1. **1**, neutrophil granulocytes; **2**, monocyte; **3**, granulocyte with no lipoid content; **4**, lymphocyte; **5**, eosinophil granulocyte.

granules; the number of these granules diminishes with the ripening of the cells, but in normal blood it is very rare to find a granulocyte devoid of lipoid granules.

SUMMARY

A short, simple and reliable histological method for staining lipoid granules in leucocytes with Sudan III is described, and a brief note is made of some of the results obtained by the application of this technique.

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A CASE SHOWING THE CHONDRO-EPITROCHLEARIS MUSCLE

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A male infant, one year old, was brought by its mother, who complained that the child had something wrong with the right arm. On examining the baby there was found a firm subcutaneous cord of tissue extending from the seventh costal cartilage to the medial epicondyle, stretching the axillary skin in the form of a web when the arm was abducted (fig. 1). The sterno-costal portion of the right pectoralis major muscle was absent. The left side was normal.

Fig. 1. Chondro-epitrochlearis muscle—right side. Age 1 year.

All shoulder movements, active and passive, were normal, but when the right hand was placed behind the back the subcutaneous band became stretched, and the child cried out with annoyance. X-ray examination showed that the clavicles, ribs, and the size of the intercostal spaces were all normal, and equal on the two sides.

Gas-oxygen anaesthesia was induced, and the band stimulated at various points throughout its length, from the chest wall to the elbow, with galvanic and with faradic current. No contraction occurred.

The child was seen one year later, and it is interesting to note that he had learned to place his hand behind his back without inconvenience by allowing the scapula to project backward, probably by relaxing the serratus anterior (fig. 2).

Testut has described two examples of the chondro-epitrochlearis muscle and one example is mentioned by Wood. Testut, Wood, Perrin and Deville considered that this fibro-muscular band has a homologue in the extensor plicae alaris of birds.

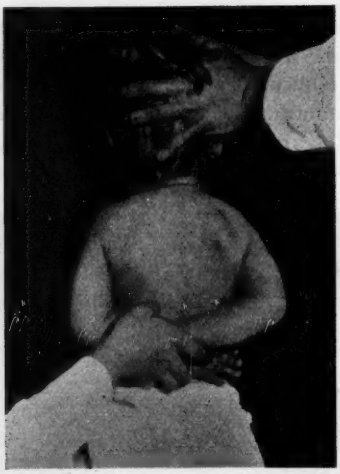

Fig. 2. Chondro-epitrochlearis muscle—age two years. Note voluntary winging of the scapula to allow the hand to be placed behind the back.

This child demonstrates the presence of a fibrous band corresponding in its position and attachments to the M. chondro-epitrochlearis, and also absence of the sterno-costal portion of the pectoralis major muscle.

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THE MORPHOLOGY OF THE LAST THORACIC TRANSVERSE PROCESS

By A. J. E. CAVE

The purpose of this note is to direct attention to the considerable uncertainty, and even actual error, which still attends the various text-book interpretations of the precise morphology of the last thoracic transverse process, an echo, possibly, of Humphrey's (1858) statement that, "the transverse processes are not quite such simple elements as they at first sight appear". And apart from their morphological aspect a certain clinical significance may attach to the various components of the neural arch, more particularly perhaps in the lower lumbar and lumbo-sacral regions, wherein, as Harris (1933) has shown, the anomalies not infrequently encountered by the radiographer demand a precise anatomical understanding for their correct interpretation.

In a "typical" thoracic transverse process, Humphrey, with reason, long ago recognised three distinct, though often confluent, tubercles (superior, anterior or external, and inferior) which, in the last thoracic transverse process, became "more expressed and distinct".

Le Double (1912) and others have given details of the degree of tubercularity of the lower thoracic and upper lumbar transverse processes. In 400 modern European skeletons the extremity of the last thoracic process was recorded as bituberculate in 25 per cent. cases, and as trituberculate in some 70 per cent. cases. Personal investigation of human material in the Hunterian Museum (Royal College of Surgeons of England) and elsewhere reveals a marked tendency towards a bituberculate condition of this process in many, if not most, non-European races, e.g. Polynesians, Papuans, Australians, Tasmanians, Bushmen, Andamanese, Javanese, Chinese, Ainos, and Negros. This bituberculate condition is effected invariably by a reduction—even to complete suppression—of the diapophysial external tubercle. In all material examined, irrespective of race and sex, the size of this external tubercle is found to be inversely proportional to that of the superior tubercle, the relative development of both tubercles being conditioned by the degree of differentiation and functional activity of the erector spinae musculature. (A very similar condition of affairs obtains in the lumbar region, again under muscular influence, in that the superior tubercle or mammillary process increases in size proportionately to the decrease in magnitude of the inferior tubercle or accessory process.) Humphrey (1858), relying upon naked-eye appearances alone, attempted the establishment of homologies between the three tubercles of the last thoracic transverse process and the tubercles upon the lumbar vertebrae. Thereby he accurately determined the correspondence between the

superior tubercle of the Th.V. 12 and the lumbar mammillary process (both metapophysial structures) and that between the inferior tubercle of Th.V. 12 and the lumbar accessory process (both obviously anapophyses). Luther Holden (1861) had also noted and figured admirably the correspondence of these parts, avoiding error by the adoption of Owen's morphological terminology. But Humphrey fell into gross morphological error in identifying the external, and wholly diapophysial, tubercle of the last thoracic with the essentially pleurapophysial "transverse process" of the loins. Now the whole weight of the evidence from embryology, human and comparative anatomy, is entirely in favour of the strictly costal nature of the lumbar "transverse process" of description. The homologue of this purely costal element in the last thoracic spinal segment is therefore, and quite obviously, the twelfth rib itself and nothing else.

Humphrey's error has been perpetuated in orthodox British anatomy through several editions of Quain (Thane, 1896; Bryce, 1914) and still obtains in Cunningham (1931) and Buchanan (1925). Some works attempt no interpretation at all of the three tubercles in question, e.g. Dixon (1912) and Gray's *Anatomy* (1932).[1] But Frazer (1920) has recognised the dubiety of existing statements and has dealt lucidly with this little problem: he figures the arrangement of the soft parts in the appropriate spinal segments, and his diagrams are confirmed by independent investigation. He has also drawn attention to the circumstance which originally led Humphrey astray—the very feeble development, or even the entire absence, of the lumbar tubercle which is the real homologue of the external tubercle on the last thoracic transverse process.

The morphological identity of the metapophysial tubercles (mammillary processes) of Th.V. 12 and L.V. 1 is clear, both having the multifidus attached; similarly the inferior tubercles of these vertebrae are purely anapophysial, giving attachment to longissimus. Dissection reveals the external tubercle of Th.V. 12 to be, not costal, but entirely diapophysial, and in all respects disposed in series with the corresponding tubercles of the remaining thoracic transverse processes. It gives attachment to the fibres of the last costo-transverse ligament, to a "levator costae" descending to the rib element of the first lumbar vertebra, and to fibres of the transverso-spinalis system. Its lumbar counterpart is a very tiny tubercle, abortive and usually suppressed, situated at the base of the descriptive transverse process, i.e. behind the morphologically costal element of the vertebra. This rudimentary structure is encountered in ideal bones only, but allowance for its potential presence is essential to the correct interpretation of homologies in the last thoracic segment of the spine, and Humphrey's failure either to recognise this little tubercle, or to accord it its proper value, was the origin of the doctrinal error in our texts.

[1] The recent, 26th, edition of Gray, however, gives (p. 214) the correct interpretation as herein set out.

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A METHOD FOR THE ORIENTATION OF RECONSTRUCTION MODELS

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The various methods now employed for accurate reconstruction from serial sections can be said to be both good and numerous. Very little, if anything, can be suggested for the improvement of the methods of Lewis (1), which he used in his classical reconstructions of the anatomy of the human embryo. More recently a more elaborate and very accurate method has been described by Heard (2), involving the use of a cine-camera. It is with some diffidence, therefore, that a new technique is put forward for the orientation of models, though it may be stated in advance that it is only applicable to certain types of series.

In reconstruction, the greatest difficulties and the greatest inaccuracies arise in the orientation of the sections cut out from wax plates or some such medium. Various methods have been devised in order to overcome these faults. They are well known and no attempt will be made to enumerate them. Some depend on the embedding, in the same block as the specimen, of some structure which will give a straight line for orientation. For this purpose pieces of liver, strands of nerve fibre, coloured grooves on the surface of the block, and many other things have been tried. In the writer's experience they are unsatisfactory, and in addition they suffer from the grave defect of giving orientation in two planes only instead of three.

The method to be described is not intended to replace the accurate devices first mentioned above. It may be of use by itself on new material, but it was primarily devised for reconstruction from a series of sections where none of the so-called aids were present. As will be seen, the only aid needed is a good photograph of the specimen before sectioning, which by most workers is obtained as a routine.

DESCRIPTION OF THE METHOD

The first essential is a good photograph of the specimen before sectioning. The photograph may be of the cleared or uncleared specimen. Either will do, but it must be distinct and clear enough to show at least two, preferably more, prominent points which can be recognised later under the microscope. For example, if the specimen is an ordinary embryo, the photograph should show with clarity such things as the optic cup, the otic vesicle, the outline of the

neural tube, the upper ends of the pharyngeal clefts, to mention only four things possible. Two points are essential, the others can be used as checks. In addition it should show the outline of the dorsal or ventral surface in the mid-line as far as is possible.

The photograph is then thrown on a screen at the magnification which is being used for the reconstruction. For convenience therefore, the specimen should always be photographed with a millimetre scale beside it. On a piece of paper pinned to the screen an outline is drawn, (*a*) of the contour of the external surface of the specimen, and (*b*) of the points, such as those indicated above, which are to be used as guides.

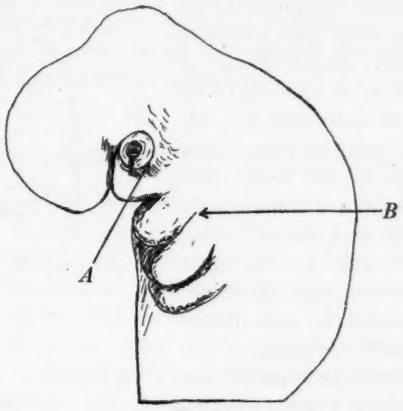

Fig. 1.

Suppose for the sake of example and illustration that the specimen to be cut is an ordinary embryo and that the drawing of the head obtained is as shown in fig. 1.

What points may be taken as guides? In this particular case the writer has found the following to be satisfactory: (1) the margin of the optic cup, (2) the place where the pontine flexure is shaved first in the sections, (3) the upper ends of the pharyngeal clefts and the angle of the mouth, and (4) the anterior end of the rhombencephalon. In a specimen of the half head of a mammalian embryo, certain points in the chondrocranium were used. Actually the points used must depend on the specimen.

In the case depicted in fig. 1, suppose that the first two points selected are the back of the optic cup, point *A*, and the upper end of the first pharyngeal cleft, point *B*. Let us assume, for the sake of example, that the sections are 10 μ thick, and that between these two points (as ascertained from examination of the series) there are seventy sections. Then the distance between the parallel planes cutting these points on the drawing must be $(700 \times M)\,\mu$, where M is the magnification. With a pair of compasses and radius $\frac{7}{10} \times M$ mm., describe a circle with centre *A*. The plane of section passing through *B* must

be a tangent to this circle, so that it can be determined and drawn in (fig. 2). Actually there are two possible tangents from point B to the circle. Inspection of the series will tell very easily which is correct.

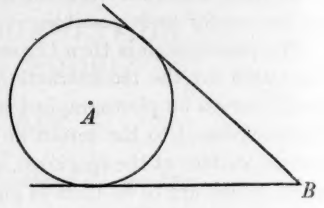

Fig. 2.

This plane of section, as seen from the lateral view, can be checked by taking other points, carrying out the same procedure, and taking an average should there be any gross discrepancy.

The next thing that must be ascertained is the degree of obliquity of the plane of section as seen from a cranial view-point. In other words, if fig. 3 represents the head of an embryo to be cut, will the plane of section lie like AB or like CD? This can be determined from certain points in the head itself without the necessity of a photograph. Suppose that in fig. 4 A and C represent the superior edges of the optic cups of the embryonic head shown in fig. 3. Let AB represent the plane of the section which just cuts the edge of the right cup, and CD the plane which just cuts the left cup, where A and C are the cups. Drop a perpendicular AE to the second parallel line. Then $AE = n \times t$, where n is the number of sections between the planes and t is the thickness of each individual section.

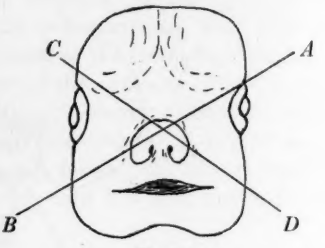

Fig. 3.

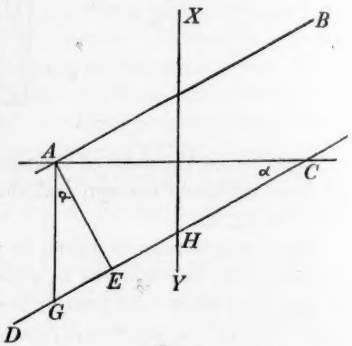

Fig. 4.

Let XY represent the mid-line of the body cut by CD at H. The distance CH is known as it can be measured on the section. The unknown quantity is the angle α, and it can be calculated as follows. Draw AG parallel to XY. Then $CH = HG$. Also the triangles AGE and AGC are similar, therefore angle $EAG = \alpha$,

$$(1)\ \frac{GE}{AE} = \tan\alpha, \qquad (2)\ \frac{AE}{EC} = \tan\alpha.$$

But $$EC = 2CH - GE.$$

Therefore $$\frac{AE}{2CH - AE\tan\alpha} = \tan\alpha.$$

Calling $CH=d$, and $AE=nt$,

$$\frac{nt}{2d-nt\tan\alpha}=\tan\alpha.$$

Therefore $nt=2d\tan\alpha-nt\tan^2\alpha$.

This is a simple quadratic equation with the only unknown being tan α, and its solution is easy.

In addition to the use of the optic cups of each side as orientation marks for the lateral obliquity, the writer has found the following points useful: (1) the lowest point and the highest point of the otocyst, (2) the junction of the submaxillary duct with the mucous membrane of the mouth, (3) the passage of the chorda tympani through the tympanic membrane, and (4) any bilateral prominent bony point. Further points will suggest themselves to any user of the method and will depend on the specimen which is being reconstructed.

THE ASSEMBLING OF THE CUT-OUT SECTIONS

The various planes of obliquity having been determined, the cut-out sections must be assembled. The obliquity of the sections in a horizontal plane can be corrected by mounting them on a board inclined at the predetermined angle α to the horizontal. In order to assemble them in proper antero-posterior relation to each other, the following method is used.

From the enlarged drawing made of the external surface of the specimen, a stencil is made in cardboard of the outline of the dorsal surface. On this section is marked out the plane of section as determined from the drawing and the position of each tenth section. When the original drawings for the reconstruction are made, there should be included in each one the dorsal (or ventral) skin surface and the mid-line of the section. And when the wax (or blotting paper) cut-outs are made these features should be incorporated in them.

The stencil is fitted into a slot on the back of the assembling board, fitted in such a way that the plane of section indicated on it lies in the same plane as the flat surface of the board. The cut-outs are then piled and stuck together in blocks of ten, care being taken that the portion of each cut-out representing the dorsal skin fits accurately into the curve of the stencil. They must, of course, be assembled so that the mid-line marked on the cut-outs coincides with the vertical plane of the stencil. The writer finds it more convenient to assemble them in blocks of ten at a time, so that any excessive thickness can be checked from the marks on the stencil and, if necessary, corrected by putting the block into a press. Finally, when the whole model is completely assembled, the stencil should fit accurately over the dorsal surface representing the skin. This can then be cut away leaving the required reconstruction by itself.

This method, clumsy and cumbrous though it may sound, has been found to give good and fairly accurate reconstructions, being particularly useful for

curved structures such as the head and the pharynx. Its defects and inaccuracies lie in the fact that it rests largely on a photograph of the specimen before embedding, when there may be marked shrinkage. If shrinkage should be uniform, this error ought to be minimal. It also rests on an assumption that the dorsal curve in the photograph represents the mid-line. This, if care is taken, will not produce a serious error. In addition, it assumes a symmetry of the two halves of the specimen. Though this is not a justifiable assumption, the error arising from it, contrasted with the usual error in reconstructions, is small and can be disregarded.

SUMMARY

A description is given of a method for the orientation of reconstruction models.

In conclusion I wish to thank Dr J. C. Tull of the Singapore College of Medicine for reading this paper in manuscript, and Mr H. L. H. H. Green of Cambridge for undertaking the proof-reading.

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APPENDIX VERMIFORMIS DUPLEX

By A. J. E. CAVE

DESPITE the notorious range of variation in characters and position natural to the human vermiform appendix, the extremes of variation—absence and duplicity—affect this organ so very rarely that every authentic example of either is worthy of record, particularly since such variations have been doubted or denied by past writers. The evidence set forth below, and submitted as additional material for the basis of a study of the underlying problem of causation, establishes beyond dubiety the morphological entity of these conditions, besides adducing a plausible embryological explanation of the cases of complete duplicity. The comparative anatomy of the caeco appendix is briefly reviewed to provide a suitable background for the consideration of the conditions occasionally obtaining in the human subject.

I. COMPARATIVE ANATOMY

Though the distinctive type of caecum with a true vermiform appendix is characteristic of the gibbons, Anthropoids and Man only, yet many sub-Primate forms exhibit a contracted caput caeci, associated more or less with the submucosal presence of lymphoid follicles of varying size, and descriptively at least termed an appendix. It must at once be emphasised that the criteria of such an organ differ somewhat in different authors' hands; that in atonic or long-preserved autopsy material it is not always easy to be sure of the existence *in vivo* of any functional appendix; that individual variation seems to play a definite role in some forms, and that it is often a moot point whether the modified caecal extremity of the lower Mammal is at all strictly comparable with the human or anthropoid vermiform process.

The presence, form and features of the caecum are largely determined by the nature of the particular diet, minor structural modifications being dependent upon peculiarities of intestinal arrangement and modes of mesenteric fixation.

In many Amphibia there occurs a pouching of the large gut at its reception of the small bowel, together with a local aggregation of lymphoid tissue. In certain Lacertilians paired caeca occur, or (as in *Iguana*) the caecum manifests a symmetrical pouching. Wood Jones (1912) has tentatively suggested that the primitive pro-mammalian caecum was a paired structure, and that the subsequent greater specialisation of the mammalian large intestine led to an asymmetry of its disposition, a shifting and kinking of the ileo-caecal angle, with the ultimate obliteration of the one and the persistence of the other of the original caecal pair. With Chalmers Mitchell (1905) he would regard the common

mammalian type of caecum as a unilateral development of the early reptilian and pre-mammalian condition of parts, seeking substantiation for this view in the presence of the double, bilocular, or subbifid caecum appearing in various Edentates, Sirenia, Rodents, and Ungulates. Similarly, on this hypothesis, the rudiment of one or other of the original pair of caecal pouches is said to be recognisable in Monotremes, Marsupials, Edentates and some Ungulates. This attractive speculation, receiving a certain support from zoology and embryology (*vide infra*), cannot be followed further here. Suffice it to say that the greatest caution is essential if one is not to endow the ordinary mammalian caecal sacculation with a morphological value in excess of its proper intrinsic nature, and that careful examination of a greater range of fresh material is desirable before the advancement of a decided opinion on this point.

The paired caecal appendages of the highly specialised Birds need small comment; assuming the form of small pouches in the raptorial species they appear as long tubular structures in the graminivorous kinds, thus affording a striking parallel to the brevity of the mammalian caecum in carnivorous species and its length and complexity in the non-carnivorous types.

Among Monotremes, *Ornithorhynchus* possesses, at the ileo-colic junction, a tubular free structure generally called caecum, provided with its own mesentery and containing much lymphoid tissue, but not differentiated into caecum proper and appendix. If a well-developed caecum be considered wanting, then this organ is the equivalent of a genuine appendix. According to MacKenzie (1916) a definite caecum and appendix occur in *Echidna* (*Tachyglossus*).

Among Marsupials the caecum is a simple undifferentiated sac (*Phalangista*, *Macropus*, *Helmaturus*, *Hypsiprymnus*, *Petrogale*, *Didelphys*), but in the wombat (*Phascolomys wombat*, *P. mitchelli*) a definite vermiform appendix is present and is subject to considerable individual variation. A similar appendix caeci is reported in *Phascolarctos*.

Among Edentates, *Orycteropus* may manifest a diminished calibre of the distal caecum; in pangolins and sloths the caecum itself is wanting; paired caeca characterise *Tatusia*, *Dasypus*, *Xenurus*, *Myrmecophaga* and *Cyclothurus*. The Cetacean caecum is a simple, bluntly rounded sac. Of the Sirenians, *Manatus* displays the paired or bifid caecum, whilst *Halichore* possesses a simple sac; neither exhibit any appendical formation.

The Ungulate caecum generally lacks any terminal differentiation, though such has been described in *Tapirus americanus*, *Babirusa*, and *Dicotyles*; in the buffaloes a subbifid caecum may occur.

An appendix in the form of a long tapering and constricted terminal caecum is present in several Rodents (*Castor*, *Myopotamus*, *Cercolabes*) but wanting in many others (*Dasyprocta*, *Hystrix* and the Sciuridae). In the hare a medial diverticulum is present additionally to the apical appendix. Probably no one of these rodent appendices is strictly comparable with the anthropoid and human appendix.

In the Carnivora the short simple caecum is usually devoid of structural

differentiation and not even a pseudo-appendix occurs in most forms: (*Canus* (dog, fox, wolf, jackal, fennec), *Nyctereutes*, *Hyaena*, *Genetta*, *Viverra*, *Suricata*, *Felis* (lion, tiger, leopard)). A descriptive appendix has, however, been reported in *Herpestes*.

The Chiroptera lack a caecum, as does *Erinaceus* among Insectivora; the Tupaiidae manifest no caecal differentiation.

The Lemuroid caecum is sacculated in *Galago*, *Loris*, and *Perodicticus*, simple and globular in *Chirogale*; its terminal portion exhibits some degree of anatomical distinctness in *Galago*, *Perodicticus* and *Chiromys*; *Nycticebus* and *Lemur* may show a well-developed appendix which is totally absent in *Tarsius* and *Loris*. An appendix has been described in *Hapale peninicillata*, in *Ateles*, and in *Lagothrix*, but *Cebus*, *Pithecia* and other New World monkeys are devoid of any such organ. In many Platyrrhine forms the termination of the conical caecum may taper to a "pointed extremity which may be elongated and resemble a vermiform appendix" (Le Gros Clark, 1934).

The absence, however, of lymphoid tissue from these so-called appendices has led Johnston (1920) to conclude that these terminal caecal prolongations are not of the nature of a true vermiform appendix.

In the Old World monkeys (*Macacus*, *Cercocebus*, *Papio*, *Cercopithecus*, *Semnopithecus*) the bluntly rounded or sometimes sacculated caecum lacks an appendix, although Wood Jones (1929) has figured it as present in a specimen of *Cercopithecus tantalus*.

The gibbons, Anthropoids and Man are characterised by the possession of a long and truly vermiform appendix arising from a capacious rounded caecum, and exhibiting a uniformity of structure, function and pathology throughout this group. Whilst the question of homologies must remain open, it is perhaps safer, in the present unsettled state of our knowledge, to regard this highest Primate type of appendix as an exclusive specialisation, lacking strict parallel among lower forms with the possible exception of Monotremes and some Marsupials.

II. CONGENITAL ABSENCE OF APPENDIX IN MAN

Two cases of total congenital absence of both caecum and appendix have been recorded, both from female subjects, the one by Robinson (1895), the other by Elliot Smith (1904). Absence of the appendix might be expected as the invariable concomitant of absent caecum were it not for the extraordinary case reported by Mouchet (1929) of a 30 years old woman in whom failure of growth and differentiation of the main caecal sac had not prevented the complete development of a typical vermiform appendix, by which alone was the first part of the large gut represented.

Cases of true congenital absence of appendix are excessively rare, as Table I indicates, only one solitary example being encountered in a specific search of over 4000 abdomens. Indeed the very existence of the condition has been denied by some (e.g. Berry, 1895), whilst Kelly and Hurdon (1905), themselves

quoting no personal case, regard all reported instances as merely examples of a secondary atrophy and obliteration, and not as genuine instances of developmental arrest.

Fawcett and Blachford's specimen was not, as Berry (1907) supposed, the sole example recorded to date, previous instances being already on record. According to Gladstone (1915) the condition was known to Meckel, and Bryant (1893) and Zuckerkandl (1894) had noted its occurrence, whilst in 1903 Huntington had published descriptions of his two specimens, the first, "a caecum having a blunt conical form without appendix", the second, a "caecum having a rounded symmetrical form, with the longitudinal muscular bands converging towards its apex, but without appendix". Gladstone himself (1915) carefully figured and described a new specimen, obtained from an elderly dissecting room female, and manifesting an "asymmetrical form of caecum without appendix".

Table I

Author	No. cases examined	Appendix absent
Treves (1885)	100	0
Ribbert (1893)	161	0
Fawcett and Blachford (1900)	350	1
Monks and Blake (1902)	641	0
Berry (1907)	100	0
Gladstone and Wakeley (1924)	3000	0
Total	4352	1

More recently Green and Ross (1933) encountered the same condition in an adult male subject, whilst Feldman (1934) recorded a right-sided caecum without appendix in a girl of 12 years, the subject of partial transposition of viscera. Though this last clinical case seems well enough founded it must be borne in mind that Bryant found an appendix of only 6 mm. in length, and Huntington one of but 5 mm.; so small an organ might easily be overlooked at operation, particularly if masked by fat, and consequently all reports from the operative field require careful scrutiny before acceptance.

To the foregoing well-established cases may be added the only specimen of its kind in the Museum of the Royal College of Surgeons of England, mentioned by Gladstone (1915) and illustrated here (see fig. 1). This specimen (Teratological Series, 549, II) is from a new-born child, the victim of epicephalocele, wherein the terminal ileum ascends parallel to and in contiguity with the medial caecal wall prior to effecting its union with the large bowel. Externally there is no demarcation of colon from caecum, nor any vestige of a caecal appendage, past or present. The case appears to be clearly one of primary congenital absence of appendix consequent upon failure of the normal caecal differentiation.

That a secondary congenital absence of appendix is, as Kelly observed, a not unknown phenomenon, seems clearly indicated by another specimen (Teratological Series, 549) in the same Museum, one collected by Sir John Bland Sutton and described in the Catalogue (and hence accepted by previous

writers) as "part of the ileum and caecum of a human foetus in which the appendix is absent and the caecum very short". Herein the ileum enters the caecum at the most dependent portion of that sac, there being no caecal bulge below the level of union of the two. But from the antero-medial aspect of the caecum an atrophied stalk-like structure ascends obliquely across the ileo-caecal junction to lie with its bulbous distal extremity closely applied to the ileal mesentery. This shrivelled pedunculated structure is undoubtedly the degenerated appendix itself, and its condition recalls very vividly those similar cases of non-pathological atrophy of this organ described by MacKenzie (1916) in the wombat (*Phascolomys mitchelli*). Some care must therefore be exercised in the examination of such specimens before a definite diagnosis of primary congenital absence is made.

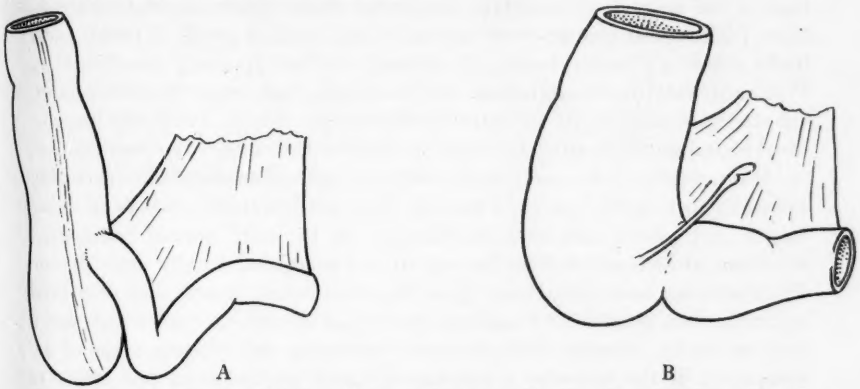


Fig. 1. A. Primary congenital absence of vermiform appendix. Mus. Roy. Coll. Surg., Teratol. Ser., No. 549, II. B. Secondary absence (congenital atrophy) of appendix. Mus. Roy. Coll. Surg., Teratol. Ser., No. 549.

One must suppose that the condition is due simply to a failure of differentiation of the primitive caecal diverticulum which makes its appearance, about the fifth week, upon the caudal limb of the extra-embryonic mid-gut.

III. DUPLICITY OF VERMIFORM APPENDIX IN MAN

Partial or complete duplicity of the vermiform appendix, though again an extremely rare condition, is nevertheless more frequently met with than the opposite extreme of total absence of that structure. The literature to date has been summarised by Greig (1934), whose excellent digest has been freely drawn upon here. Additional instances to those already published might be cited did they not emanate from the relatively uncritical field of operative surgery and in consequence lack the requisite criteria of proof.

Descriptively, known examples of appendix duplex may be resolved into the following three types:

Type A. Single caecum with one appendix exhibiting partial duplicity.

Type B. Single caecum with two obviously separate appendices (complete duplicity).

Type C. Duplicity of caecum, each caecum bearing its proper appendix.

The simplest illustrations of the first type are those curious specimens of "double-barrelled" appendix wherein the single organ presents two distinct lumina throughout either its entire length or throughout only a part thereof. Rosenberger (1903) has described such an example from an adult male, the two lumina sharing a common muscular and serosal investment. Prentiss (1907) apparently encountered a similar specimen, though its structural details are not forthcoming. Later, Elwyn (1924) discovered at operation a two-limbed appendix which he regarded as representing two originally entire organs which had subsequently undergone fusion of their distal extremities. Proximally each limb of the anomalous formation manifested characteristic appendical structure; pathological changes were absent; distal to their point of fusion both limbs shared a common lumen. A curiously divided appendix was found by Walthard (1931) in a female infant. After a normal single origin from the caecum, the structure divided into two parallel tubes which, sharing a common longitudinal musculature, reunited distally to re-establish their original common lumen.

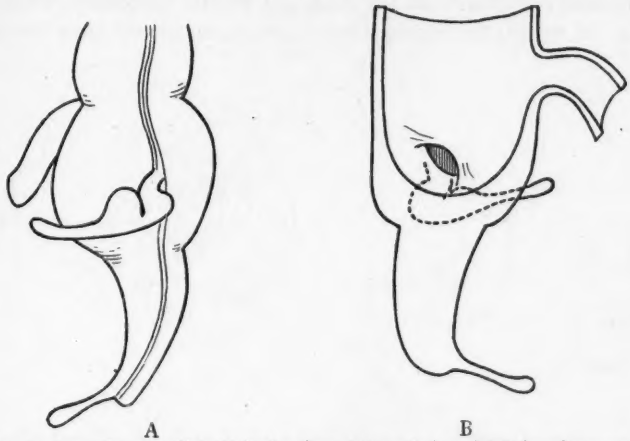
More recently Clavel and Colson (1933) encountered an extraordinary double appendix in a thirty years old woman. Two contiguous appendices of equal length and calibre, and each manifesting the typically normal histological structure, arose together from the caecum and proceeded distally some 70 mm. Their separate and distinct tips gave the macroscopic appearance of a bifid appendix, but apart from a common peritoneal investment each organ was a distinct entity. Whether their specimen represents the ultimate stage of development of the bilocular condition exhibited by the cases just cited, or whether it is here a question of the subsequent juxtaposition of two originally separated processes, is not easily determined; if the latter view be held concerning this anomaly then the case should be transferred to type B.

It is more than likely that the non-pathological diverticulum of the appendix described by Walmsley (1929) represents an attempted bifurcation or duplicity of this organ and belongs to the first type described here. The writer has seen a very similar case secured at operation and unfortunately not preserved, wherein the diverticulum exceeded in proportions that figured by Walmsley, and the whole appearance of the specimen was that of a bifid appendix.

Of the specimens grouped under type B the earliest undoubted example was that recorded by Paterson and Emrys-Roberts (1906), where in a full-time foetus the subject of ectopia viscerum, spina bifida, and other congenital anomalies, a "small sacculated and curved appendix" lay on each side of the ileo-caecal junction. Subsequent cases occur in the clinical rather than the anatomical literature. Thus Schooler (1907) encountered at operation a 100-mm. normal appendix accompanied by a second and smaller fellow, which arose from the caecum some 20 mm. below the first, and which had perforated as the result of acute inflammation.

Young (1911) likewise met with two almost equally long appendices arising some 30 mm. apart from the caecum of a 21 years old female, each possessing its own mesentery and each the seat of purulent inflammatory change. According to Bérard and Vignard (1914) Jalaquier had operative experience of a double appendix, whilst Braatz (1929) appears to have encountered another example in a young adult female. In a 14 years old boy Goldschmidt (1930) found a supernumerary appendix arising from the anterior wall of the caecum.[1]

To these recorded instances may be added the present example which, like the first case quoted, hails from the anatomical laboratory and with that case establishes beyond cavil the entity of appendix duplex. The specimen (fig. 2)



A B

Fig. 2. Appendix vermiformis duplex from (A) ventral, and (B) dorsal aspects.

was obtained some years ago from an otherwise unremarkable full-time foetus by Mr H. C. Wilson, Prosector to the Royal College of Surgeons' Museum. The terminal ileum enters the large bowel at the customary angle, and the caeco appendix retains its infantile form. The caecum proper comprises a proximal dilated moiety succeeded immediately by a constricted conical distal moiety, from the left side of the apex of which a 15-mm. long vermiform appendix proceeds infero-medially. This appendix has now lost any original mesentery it may have possessed; it communicates freely with the caecal cavity and does not appear to be furnished with a valve of Gerlach. On the postero-medial wall of the proximal caecal segment an orifice, guarded by prominent crescentic folds of the mucosa, leads into the lumen of a second vermiform appendix. This supernumerary appendix, bound to the caecum by a short mesentery and angulated soon after its commencement, is some 20 mm. long; it has a calibre at least twice that of the apical appendix, and, save for its tip, is entirely retro-

[1] Dr D. E. Robertson, of Toronto, informs the writer (12 November, 1935) that he has seen in Boston "a specimen of a caecum that has two vermiform appendices". This case is described by Dr H. M. Pratt in the *Amer. J. Dis. Child.*, June 1933, vol. XLV, pp. 1263–76.

caecal. One of the taenia coli may be traced to the roots of both appendices, which lie about 15 mm. apart. Both structures manifest an identical normal histology.

In the interpretation of this interesting specimen the apical vermiform process is regarded as the equivalent of the normal appendix, which at this age and for some time thereafter displays such a terminal disposition. The retrocaecal appendix is supernumerary, and its presence is to be explained by the persistence and development of a fugitive embryological structure, of great morphological interest, but one commonly ignored in standard embryological works. This structure is the "transient appendix" which Kelly and Hurdon (1905) described in their study of the developing caeco appendix in over fifty young human embryos from the Mall and Brödel collections. They figured (*op. cit.* p. 72, fig. 28) this curious and temporary outgrowth from the tip of the

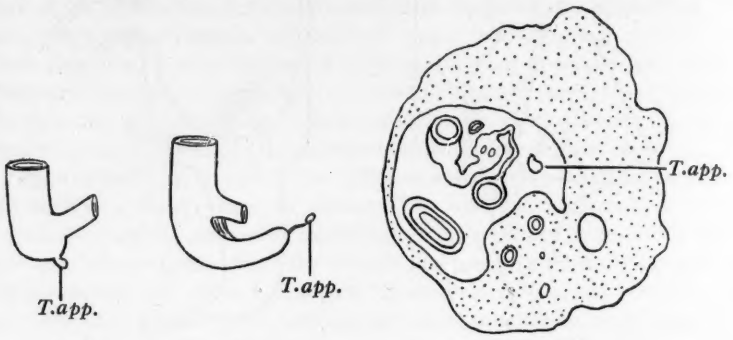


Fig. 3. The "transient appendix", (A) at the sixth week, (B) at the seventh week (after Kelly and Hurdon), (C) at the 20-mm. stage (after Gladstone).

caecum, growing during the sixth week and atrophying towards the end of the seventh week or very soon after (see fig. 3). Gladstone (1924) confirmed the presence and nature of this "transient appendix" in 10–20 mm. embryos, and has figured its appearance in a cross-section of the umbilical coelom at the 20-mm. stage. He describes this curious process as "a remarkable outgrowth from the tip of the caecum. . . which simulates in its position and form the true vermiform appendix", but adds, rightly, that, "since it atrophies in embryos of 20 mm. length, and afterwards completely disappears, it is believed to be an independent structure, and not connected with the permanent appendix caeci, which is differentiated later". This clear statement requires no elaboration. Suffice it here to remark that in the "transient appendix" lies a definite potential embryological origin for the development of a supernumerary appendix, and the most plausible explanation of the specimen of appendix duplex herein described as well as of the cases quoted from the literature. Whether the presence of the "transient appendix" may be regarded as affording substantiation of the theory of an ancestral caecal duplicity in the Mammalia is a

morphological question beyond the scope of this paper; for its solution there is not, as yet, a sufficiency of data from comparative vertebrate embryology.

Under type C two cases may be quoted for the sake of completeness. In a 10 weeks old female Greig (1934) found the whole bowel duplicate distal to the site of Meckel's diverticulum; two separate caeca were present, each bearing its proper vermiform appendix.

Fig. 4. Duplicity of ileum, caecum and appendix.
Mus. Roy. Coll. Surg., Teratol. Ser., No. 548, D *a*.

A specimen (fig. 4) from the Teratological Series of the Royal College of Surgeons' Museum (No. 548, D*a*) illustrates a not dissimilar duplicity of the parts derived from the apex of the primitive mid-gut loop, wherein the caecum and appendix are necessarily involved. The specimen is from a new-born infant, the subject of umbilical hernia. The ileum bifurcates so that its last 17 cm. or so consists of two separate and symmetrical tubes, each of which joins a separate caecum. The caeca are adherent mesially and each is provided with its own vermiform appendix; distally they unite to form a single ascending colon completely divided by an internal septum of mucous and submucous tissue.

Concerning such bizarre duplicity of the bowel no very satisfactory embryo logical explanation is as yet forthcoming, and further examples need not therefore be quoted.

SUMMARY

Though a remarkably constant structure in Man, the vermiform appendix is nevertheless occasionally subject to the extremes of variation, i.e. total suppression and duplicity. Cases of appendix duplex are described and are seen to fall into two categories: (*a*) supernumerary appendix due to persistence of a transient embryological structure; (*b*) appendical duplicity incidental to a more general affection of the primitive mid-gut.

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COMPARISON OF THE LATERO-SENSORY LINES, THE SNOUT AND THE CRANIAL ROOFING BONES OF THE STEGOCEPHALI WITH THOSE IN FISHES

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In my recent work "On the general pattern of arrangement of the cranial roofing bones in Fishes" (Allis, 1935), I was led to suggest that the latero-sensory components of the cranial roofing bones of Fishes had been gradually suppressed in the Amphibia together with the latero-sensory lines as these animals took more and more to life on land. That they had primarily possessed these bones, inherited from their fish ancestors together with the latero-sensory lines, did not occur to me to doubt, but I did nevertheless question the presence of these bones as independent and important elements of the cranial roofing bones of the Arthrodira. My present work leads me to doubt their presence also in the cranial roofing bones of the Amphibia, and as this opinion is based largely on the manner of development of the latero-sensory lines, what is actually known regarding this in the recent Fishes and Amphibia and what may be reasonably assumed regarding it in their ancestors must first be considered.

The several latero-sensory lines of Fishes first appear, as is well known, as strands of special tissue developed in connection with certain of the ectodermal placodes, these strands lying directly upon the basal membrane of the ectoderm and being covered superficially by one or more layers of its cells. Each strand is connected at intervals by branches with the nerve of the line, and at these points the primary organs of the line arise. The strand and the related organs thus lie primarily wholly in the ectoderm, the organs exposed on its outer surface, but this condition does not usually persist in the adults of recent Fishes and amphibians, the organs there usually becoming enclosed either in sac-like pits, in open grooves or closed canals, the formation of these grooves and canals taking place in what is apparently two distinctly different ways, as explained immediately below.

In *Petromyzon* each organ of the cranial lines is said by Langerhans (1873) and Tretjakoff (1916) to lie at the bottom of a short and slit-like groove on the outer surface of a small pillow-like elevation of the dermis, the bottom of the groove lying approximately in the level of the general outer surface of the ectoderm. Each cranial line accordingly presents the appearance of a series of short and slit-like grooves, as shown by Leydig (1879) in a figure reproduced in the accompanying fig. 1A. In larvae these grooves are said

by Langerhans to be considerably deeper than in the adult, and the walls of the grooves are shown by him to be formed by a definite infolding of the entire ectoderm, as shown in the accompanying fig. 1B. Tretjakoff, however, says that in all the larvae (Ammocoetes) examined by him the grooves were but slightly deeper than in the adult. In the Myxinoidae the latero-sensory lines probably resemble those in *Petromyzon*, but I do not find them described. The Cyclostomata are generally considered to be direct descendants of the Ostracodermi, and because of the character of their snouts, Stensiö (1927) considers the Petromyzontidae to be descendants of the Osteostraci, and the Myxinoidae to be descendants of the Heterostraci. If, however, the latero-sensory lines are similar in these two orders of the Cyclostomata, it seems to me much more

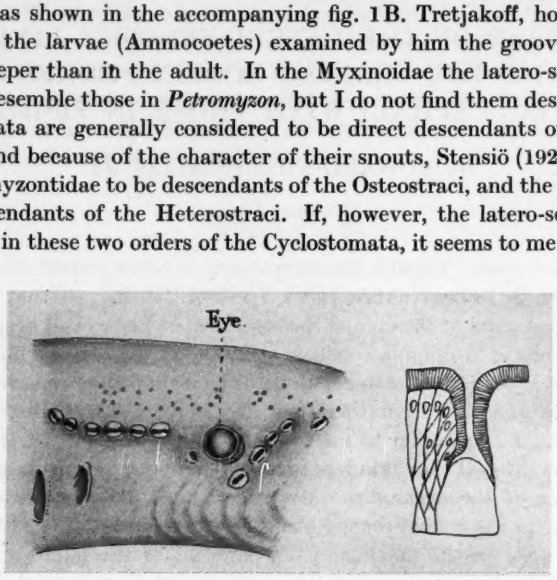

Fig. 1. A. Lateral view of part of the head and body of *Petromyzon marinus*. After Leydig. B. Transverse section through a cranial latero-sensory organ of an Ammocoetes of *Petromyzon planeri*. After Langerhans.

probable that they are both descendants of the Osteostraci. In *Cephalaspis*, which is one of the latter Fishes, the positions of the latero-sensory lines are considered to be indicated by slight scratches on the outer surface of the dermal cranial shield (Stensiö, 1927). These scratches must have lain directly beneath short sections of latero-sensory canals, short slit-like grooves resembling those in *Petromyzon*, or short lines of pit organs similar to those in certain of the recent Teleostomi, and the evident relationship of *Cephalaspis* and *Petromyzon* strongly suggests the slit-like grooves of the latter fish. The Osteostraci and Cyclostomata thus form a group of Fishes in which the organs of the cranial latero-sensory lines have apparently retained, throughout phylogeny, their primitive positions wholly in the ectoderm and exposed on its outer surface, and they are the only Fishes in which this can be assumed to have taken place.

In the adults of the recent Elasmobranchii the latero-sensory lines either lie along the bottoms of open grooves or are enclosed in closed canals, and these canals are certainly developed in two distinctly different manners in the Plagiostomi and Holocephali. In the Plagiostomi each of the organs of a line

that will later become enclosed in a canal sinks, at a certain period of development, somewhat below the external surface carrying with it cells that have been proliferated from adjacent portions of the ectoderm. The layer of ectoderm that lies directly above the organ then breaks down and a little sac-like pit is developed, the bounding walls of which are of ectodermal origin. There is here no slightest indication of any infolding of the entire ectoderm, for if there had been any there would have been folds or creases on the outer surface of the ectoderm radiating from the external opening of the pit and there is no slightest indication of them. As the organ thus sinks below the ectoderm, the strand of special tissue that connects it with other organs of the line also becomes detached from the overlying layers of the ectoderm and sinks inward, thus forming a solid cord of tissue which connects each two adjacent pits of the line, and in this cord the continuous canal later develops. There is thus here no possible question of any infolding of the entire ectoderm such as takes place in the formation of the slit-like grooves of *Petromyzon*. In some of these Fishes parts of certain of the latero-sensory lines lie in open grooves, instead of in closed canals, but the development of these grooves has never been described, so far as I can find. In *Chlamydoselachus* (Allis, 1923*a*) I have shown these grooves, certain of them forming direct prolongations of parts of the lines that are enclosed in canals, and in such cases the floor of the groove lies in the level of the floor of the canal to which it is related. It therefore seems quite certain that each of these grooves has been developed, as the canals have, by the sinking inward of the entire related latero-sensory line, but that here the layer or layers of the ectoderm that lay superficial to the line have broken down between successive organs as well as directly superficial to the organs themselves. That the open groove could have arisen by an infolding of the entire ectoderm, while the closed canal with which it is directly continuous develops in a totally different manner, seems wholly improbable. The edges of the grooves of *Chlamydoselachus*, it is to be noted, are serrated.

In *Chimaera*, one of the Holocephali, the latero-sensory lines all lie in open grooves, and it is quite certain that these grooves were developed as they are in *Chlamydoselachus*, and hence probably in the manner just above assumed for the latter fish. The edges of the grooves are, however, here straight instead of being serrated, thus leaving a narrow slit-like opening leading into the groove. Near the anterior ends of the lines these slit-like openings become somewhat enlarged between each two adjacent organs of a line, thus forming primary pores leading directly into the grooves. The walls of the grooves are said to be strengthened by a series of incomplete rings of a material that Solger (1880) refers to as bone-like, while Cole (1896) refers to it as semi-cartilaginous. In *Callorhynchus*, another of the Holocephali, the open grooves of *Chimaera* have become closed canals by the meeting and coalescence of their edges, primary pores being left leading into the canal between each two adjacent organs of a line.

In the non-Macropetalichthyid Arthrodira, the latero-sensory lines lay in open grooves on the outer surfaces of the dermal cranial bones, and cross-sections through these grooves markedly resemble in shape those through the grooves of *Chimaera*. It is therefore exceedingly probable that these grooves were open to the exterior throughout their entire lengths, as they are in *Chimaera*. There is no slightest indication of independent bones similar to the latero-sensory bones of the recent Teleostomi having been developed in protective relation to these canals, the grooves apparently corresponding to, but being much deeper than, the open latero-sensory grooves of the Labyrinthodontia. In the Macropetalichthyid Arthrodira these open grooves are replaced by closed canals, and Stensiö (1925) describes in *Epipetalichthys* a single row of small unbranching tubules leading into each of these canals, while in *Macropetalichthys* there are two parallel rows of tubules leading into each of the canals, these tubules being said to be much more numerous in each of these Fishes than the organs in the corresponding parts of the canals. These closed canals were certainly preceded ontogenetically by open grooves, and it is probable that the edges of these grooves were serrated as they actually are in *Chlamydoselachus*. When the edges of these grooves met and coalesced to form the closed canals it is therefore probable that these little tooth-like projections met and coalesced with each other end to end in *Epipetalichthys*, thus there giving origin to a single row of tubules leading into the canal, while in *Macropetalichthys* the little tooth-like projections interlocked to a certain extent before coalescing, thus there giving origin to the two parallel rows of tubules. The canals of these Fishes, like those in the Holocephali, are thus quite certainly not the homologues of those in the recent Plagiostomi, and it is probable that the canals were of this same type in the Heterostraci, for in *Pteraspis* there are said to be numerous small unbranching tubules leading into them.

In the recent Crossopterygii, Dipnoi and Actinopterygii, the canals are developed, so far as is known, in a manner that is in principle strictly similar to that described by me in *Amia* (Allis, 1889). A short groove develops in relation to each organ of a line, the edges of the groove projecting outward pillow-like as they do in *Petromyzon*, but the edges of these pillows here arch over the organ and coalesce, thus enclosing it in a short canal. These short canals then increase in length and meet and coalesce end to end with adjacent and similarly developed short canals of the line, a continuous canal thus being formed with a single primary pore or tube leading to the exterior between each two of the canals. The walls of the canals are thus formed by a definite infolding of the entire ectoderm, the canals differing markedly in this respect from those in the Heterostraci, Arthrodira and Elasmobranchii. The individual organs of the lines always remain definitely in the ectoderm, so far as I recall, never sinking beneath it to form little sac-like pits such as develop in the Plagiostomi preliminary to the formation of the continuous canals. Furthermore, these are the only recent Fishes in which primarily independent bones are ever developed in protective relation to the latero-sensory canals, for the semi-

cartilaginous incomplete rings that partly surround the open grooves in *Chimaera* can certainly not be compared with the latero-sensory bones of the recent bony Fishes. The manner of development of the canals in the fossil Crossopterygii, Dipnoi and Actinopterygii is of course unknown, but as independent latero-sensory bones develop in all these Fishes in protective relation to parts of certain of the canals, it seems proper to assume that the canals of these fossil Fishes were developed, as in the recent bony Fishes, by a definite infolding of the entire ectoderm.

The latero-sensory lines of Fishes thus indicate three lines of descent, one represented by *Cephalaspis* and the Cyclostomata, a second by *Pteraspis*, the Arthrodira and Elasmobranchii, and a third by the Crossopterygii, Dipnoi and Actinopterygii, this third line lying between the other two but related to the *Cephalaspis*-Cyclostomata line rather than to the Arthrodira-Elasmobranchian one.

The ampullary organs and nerve sacs are not here taken into consideration, for they take no part in the formation of the several main lines, and there is furthermore question as to their homology. They are found only, so far as I recall, in the Elasmobranchii and the recent Chondrostei, which would seem to be favourable to Sewertzoff's (1926) contention that the Acipenseridae are of ancient origin and related to the Elasmobranchii, but so far as can be judged from his descriptions of early embryos of *Acipenser ruthenus*, the latero-sensory canals of this fish are developed by an infolding of the entire ectoderm, as they are in *Amia*.

The Stegocephali may now be considered, and as it is the character of the latero-sensory lines and not minor variations in the number and arrangement of the cranial roofing bones that is important in the present discussion, I shall refer only to Säve-Söderbergh's 1932 description of the Ichthyostegalia, his 1935 work on this same subject not being at my disposal, and to Moodie's (1908, 1916) descriptions of the Labyrinthodontia; and I shall assume that there are no latero-sensory components in the cranial roofing bones. The names given by these authors to the several bones will be employed in the descriptions, but I shall frequently give after these names, but enclosed in brackets, the names of the bone or bones of Fishes that I consider to be their homologues. The olfactory perforations of the dermal cranial shield will be called the nasal openings, the term nasal apertures being reserved for the openings on the external surface of the skin.

ICHTHYOSTEGALIA

These Stegocephali have been found only in the Upper Devonian rocks of Greenland and are the earliest known of all the Amphibia. They have been carefully described by Säve-Söderbergh (1932) and in them the latero-sensory lines are enclosed in the dermal cranial bones, they being the only amphibians in which this is known to have taken place.

In *Ichthyostega*, which is one of these amphibians (figs. 2 and 3), the external nasal openings, one on either side of the head, are shown lying wholly on the

ventral surface of the skull, and that part of this ventral surface on which they lie seems quite unquestionably to be homologous with what Stensiö (1934) has called in *Pholidosteus* the rostral face and which I have described in certain of the Arthrodira and fossil Chondrostei as the antero-ventrally presented anterior end of the skull (Allis, 1934). This surface, which is evidently the ventral surface of the snout, is, however, presented much more ventrally in *Ichthyostega* than in the Fishes above referred to.

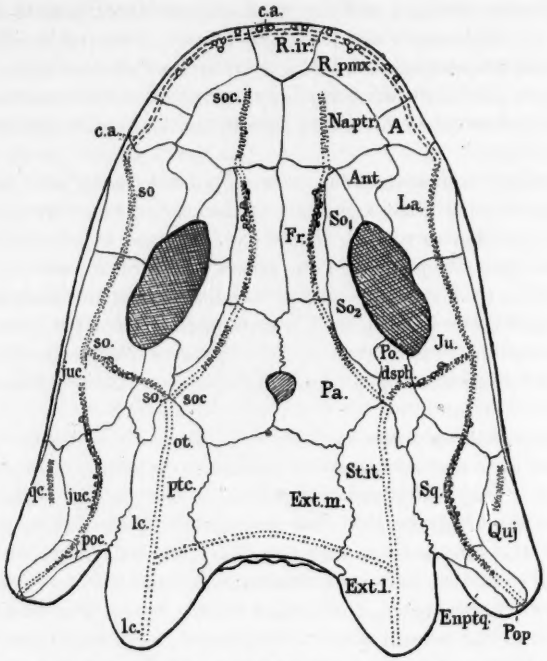

Fig. 2. Dorsal view of the skull of *Ichthyostega* showing latero-sensory lines. After Säve-Söderbergh.

The rostral face of the snout, as above defined, is completely covered on either side from the anterior edge of the external nasal opening to the median line by the premaxillary part of a bone called by Säve-Söderbergh the rostro-premaxillary, this part of the bone bearing the premaxillary teeth along its ventro-posterior edge and fusing completely in the median line with its fellow of the opposite side. The rostral portion of the bone lies on the dorsal surface of the snout along its antero-lateral margin, and has on its outer surface the characteristic surface markings of the dermal cranial bones. It lies approximately at right angles to the premaxillary part of the bone and has fused completely with the dorsal edge of the postero-lateral two-thirds of that part of the

bone. It articulates postero-laterally with a small so-called anterior antorbital, postero-mesially with a naso-postrostral and antero-mesially with the lateral edge of a small median so-called rostro-interrostral. The latter bone lies on the dorsal surface of the anterior end of the snout, its anterior edge lying upon the dorsal edges of the mesial thirds of the premaxillary portions of the rostro-premaxillaries of opposite sides. This little bone thus corresponds to the one or two median dorsorostrals of certain of the Arthrodira and fossil Chondrostei and Crossopterygii completely fused with each other but not bent downward over the anterior edge of the snout on to its rostral face, the rostral part of the so-called rostro-premaxillary apparently corresponding to certain of the lateral dorsorostrals of *Polyodon* and *Acipenser* (Allis, 1935).

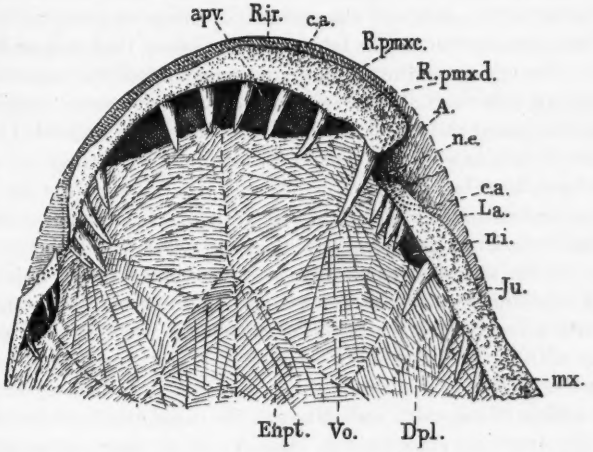

Fig. 3. Ventral view of the skull of *Ichthyostega eigili*. After Säve-Söderbergh.

The anterior edge of the rostro-interrostral (median dorsorostral) and the antero-lateral edge of the rostral part of the rostro-premaxillary (dorsorostro-premaxillary) are each perforated by a line of small unbranching vertical canals, and Säve-Söderbergh considers them to have been traversed by primary tubes leading downward to an ethmoidal cross-commissural latero-sensory canal. The latter canal is said to have lain along the rostral face of the snout in a groove that lies between the dorsal edges of the premaxillary portions of the rostro-premaxillaries and the overlying ventral surfaces of the rostro-interrostral and the rostral portions of the rostro-premaxillaries. It was not enclosed in any bone that has been preserved in the fossil. There were accordingly in these amphibians no bones at all comparable to the so-called rostrals, interrostrals or lateral rostrals of Stensiö's descriptions of the fossil Chondrostei and Crossopterygii, for these bones always enclosed parts of the ethmoidal canal and were quite unquestionably developed in direct protective relation

to that canal. It is however possible that the canal of *Ichthyostega* was enclosed in a delicate tube of bony or semi-bony material similar to that which is shown in Stensiö's (1921) figures of *Axelia* enclosing the postorbital and suborbital portions of the main-infraorbital canal and possibly also enclosing that part of the supraorbital canal that lay between the frontal and the supraorbitals. There are along each half of the canal of *Ichthyostega* eight primary tubes, and notwithstanding that the canal lies on the ventral surface of the snout, the tubes lead upward and open on its dorsal surface, which would seem to indicate that the canal was here in process of passing either from the dorsal to the ventral or from the ventral to the dorsal surface of the snout. If the canal were in process of passing from the dorsal to the ventral surface of the snout, it might offer an explanation of the unusual fact that the bones traversed by these tubes lie between the canal and the external openings of the tubes and hence are in no way comparable to the latero-sensory bones that enclose the canals in the recent bony Fishes. Furthermore, it seems improbable that there could have been along this short stretch of canal eight latero-sensory organs similar to those in *Amia* and the recent bony Fishes, that is, an organ between each two primary tubes, and if the organs were less numerous than the tubes the canal could not have been enclosed in the manner described by me in *Amia*.

Posterior to the rostro-interrostral and to the rostral portion of the rostro-premaxillary there is, on either side, a bone called by Säve-Söderbergh the naso-postrostral which articulates mesially with its fellow of the opposite side, articulates posteriorly with the frontal and anterior supraorbital, postero-laterally with a bone called the posterior antorbital and laterally by its small lateral edge with the so-called anterior antorbital. The supraorbital canal, after traversing the frontal close to its lateral edge, enters the naso-postrostral at about the middle of its width and although the canal could not be traced forward in this bone Säve-Söderbergh considers it to have extended forward to the anterior edge of the bone. Comparing this bone, as thus described, with those in the corresponding position in Fishes, excepting the Arthrodira, the bone of *Ichthyostega* would certainly be considered to contain a latero-sensory nasal bone fused with a posterior inner lateral dorsorostral and probably also with a small posterior outer lateral dorsorostral. If however, as I have assumed, there are no latero-sensory bones in these amphibians, this bone must have been formed by the fusion of an inner lateral and an outer lateral dorsorostral.

The so-called posterior antorbital of *Ichthyostega* is evidently either the preorbital or simply the posterior one of the two preorbitals of my descriptions of Fishes, and the so-called anterior antorbital either an anterior preorbital or a bone that does not occur as an independent bone in the Fishes considered by me in my recent work. The latter bone lies on the dorsal surface of the nasal capsule, forms the dorsal boundary of the external nasal opening and is considered by Säve-Söderbergh to probably represent the septomaxillary of the recent Amphibia.

Posterior to the inner lateral dorsorostral component of the naso-postrostral

(posterior dorsorostral) there is a bone called by Säve-Söderbergh the frontal, posterior to that a fronto-parietal and posterior to the fronto-parietals of opposite sides a median parieto-extrascapular, and regarding the homologies of these several bones as compared with those in Fishes there is some question. The so-called fronto-parietal, which is index-lettered *Pa* in Säve-Söderbergh's figure, encloses between itself and its fellow of the opposite side the parietal (pineal) foramen, and the corresponding bone in others of the Stegocephali is currently called the parietal, Säve-Söderbergh's parieto-extrascapular being called either the supraoccipital, dermo-supraoccipital, postparietal or interparietal. In all of the Fishes considered in my earlier work the pineal foramen, when definitely shown, always lies between the frontals of opposite sides, but in *Coccosteus* a bone called the pineal plate lies between the anterior ends of the frontals. The position of the pineal foramen in *Ichthyostega* between the so-called fronto-parietals is doubtless in part what has led Säve-Söderbergh to give this name to these bones, the frontal component of each bone being considered to extend posteriorly to a line slightly posterior to the parietal foramen. That this anterior part of the bone of *Ichthyostega* does actually correspond to part of the frontal of Fishes is still further indicated by the course of the supraorbital canal, which after traversing the frontal close to its lateral edge traverses in similar position that part of the fronto-parietal that Säve-Söderbergh considers to belong to the frontal. It seems to me much more probable, however, that the frontal and the entire fronto-parietal of *Ichthyostega* together correspond to the frontal of Fishes and the presence in Säve-Söderbergh's figure of *Dipterus* (1932, p. 96) of two so-called medial frontals, one lying posterior to the other, is in favour of this view. If this be correct, the frontal of *Ichthyostega* and hence the anterior one of the two medial frontals of Dipterus would probably correspond to the frontal of higher Vertebrates, the fronto-parietal of *Ichthyostega* and the posterior one of the two medial frontals of *Dipterus* corresponding to the parietal. The so-called parieto-extrascapular of *Ichthyostega* would then correspond to the median parieto-extrascapular of Fishes and the parietal part of it alone, or the entire bone, would correspond to the dermo-supraoccipital of higher Vertebrates, this depending upon whether the extrascapular bones of Fishes disappear or persist in higher Vertebrates.

Postero-lateral to the posterior portion of the parieto-extrascapular there is a lateral extrascapular that is strictly comparable in position to the similarly named bone in Fishes, and anterior to it there is a bone that articulates mesially with the anterior portion of the parieto-extrascapular and with that part of the fronto-parietal (posterior frontal) that lies posterior to the parietal foramen. Because of these topographical relations to what Säve-Söderbergh considers to be the parietal components of the latter two bones he considers it to correspond to the supratemporal and intertemporal of Stensiö's (1922*a*) descriptions of *Eusthenopteron* fused with each other, and accordingly calls it the supratemporo-intertemporal. There is, however, question as to the homology

of these two bones of *Eusthenopteron*, and in my recent work (Allis, 1935) I concluded that they must be, respectively, posterior and anterior dermopterotics. Anterior to the so-called intertemporal of *Eusthenopteron* there is a dermosphenotic in typical relation to the adjacent bones and to the main infraorbital and supraorbital canals. In *Ichthyostega*, on the contrary, the supraorbital canal anastomoses with the main-infraorbital immediately after the latter issues from the anterior end of the so-called supratemporo-intertemporal, the hind end of the supraorbital canal here being wholly enclosed in a projecting corner of the fronto-parietal (posterior frontal). That part of the main-infraorbital canal that lies anterior to this point of anastomosis turns antero-laterally and enters and traverses a bone called by Säve-Söderbergh the postorbito-dermosphenotic. The conditions here thus strongly suggest those in *Amia* and *Polypterus* rather than those in *Eusthenopteron*, and I accordingly consider the so-called postorbito-dermosphenotic to be a suprapostorbito-membranosphenotic, the dermosphenotic as an independent bone being wholly wanting.

Anterior to the postorbito-dermosphenotic (suprapostorbito-membranosphenotic) there are posterior and anterior supraorbitals, the latter extending forward to the hind edge of the naso-postrostral and articulating laterally with the posterior antorbital (posterior preorbital), the latter bone forming the larger part of the anterior boundary of the orbital opening.

The main-infraorbital canal after traversing the postorbito-dermosphenotic (suprapostorbito-membranosphenotic) enters and traverses a so-called jugal which is postorbital and suborbital in position, beyond which it enters a lachrymal and could be traced to about the middle of the length of that bone where it disappeared in a group of tubules. Beyond that point the canal could not be traced, but Säve-Söderbergh considers it to have continued onward in the bone to its lateral edge not far from its anterior end and there to have turned downward behind the external nasal opening and then forward beneath it. Beyond this it turned upward and forward across the external surface of the hind end of the premaxillary portion of the rostro-premaxillary (dorsorostro-premaxillary) to reach the groove already referred to that lies between the dorsal edge of the premaxillary portion of the rostro-premaxillary and the under surfaces of the rostral part of the latter bone and the rostro-interrostral (median dorsorostral). There is no indication that the canal after leaving the lachrymal was anywhere enclosed in bone, thus apparently lying in the dermis throughout this part of its course. The lachrymal articulates anteriorly with the small so-called anterior antorbital (anterior preorbital) and apparently forms part of the posterior boundary of the external nasal opening. Ventrolaterally it is in articulation with the maxillary.

The internal nasal opening is bounded mesially by the vomer and laterally by the maxillary, and in a figure giving a ventral view of the skull and reproduced in the accompanying fig. 3, it is shown lying wholly posterior to the external opening. The line of the maxillo-premaxillary teeth passes between

the two openings, as does also the line of the main-infraorbital latero-sensory canal. Whether this latter canal passed internal or external to the naso-buccal (choanal) canal is of course not known, but if it passed internal to it, as the corresponding canal does to the naso-buccal groove in those of the Plagiostomi in which there is an internal nasal aperture, the conditions here would be so markedly similar in these two groups of vertebrates that the internal nasal apertures must be homologous. If then this internal nasal aperture of the recent Elasmobranchii was inherited directly from Devonian ancestors, it would be a feature in which the Ichthyostegalia show affinity to the Arthrodira-Elasmobranchian line.

The probable character of the latero-sensory canals of this amphibian may now be considered. According to Säve-Söderbergh the bottom of each canal presents a series of depressions, each of which is considered by him to have lodged a sense organ of the line, and between each two of these depressions and hence between each two adjacent organs of the line, a primary pore or simple unbranching primary tube is said to lead from the canal to the outer surface of the bone. Säve-Söderbergh accordingly concludes that the canals of the adult Ichthyostegalia correspond to that stage in the development of the canals of *Amia calva*, as described by me (Allis, 1889), in which the primary pores and tubes have just been formed but have not yet undergone dichotomous subdivision. This would however offer no explanation either of the eight unbranching tubules leading into each half of the ethmoidal canal or of the group of tubules said to arise from the main-infraorbital canal at about the centre of the lachrymal bone, for in both of these places it seems quite certain that there must have been more than one tubule between each two organs of the line. The conditions described in these amphibians would however have been acquired if the canals were developed as I have assumed them to have been in the Macropetalichthyid Arthrodira, and I consider this to be the case. These Stegocephali would then be related in this respect, as well as in the possession of an internal nasal aperture, to the Arthrodira-Elasmobranchian line of descent instead of to the Crossopterygian, and the character of the snout and the external nasal openings are also in favour of this assumption.

The development of the snout is known in only a few of the recent Ichthyopsida, and even in them it is not fully known. It is however known, as fully discussed in several of my works (Allis, 1923*b*, 1931*a*, 1931*b*, 1932), that in early stages of development of all these vertebrates the snout is of what I have called the trabeculo-cranial type, the nasal pits and the ethmoidal latero-sensory line, if present, lying on its ventral surface slightly anterior to the bucco-pharyngeal upper lip. The latter lip, usually called by other authors the hypophysial and posthypophysial bolster, lies at the dorso-anterior edge of the oral plate and forms the posterior boundary of the point of attachment of the hypophysial cord or canal to the external ectoderm. This lip is actually what remains of the ectodermal and immediately underlying tissues that primarily covered the premandibular visceral arches and the anterior surface

of the premandibular portion of the alimentary canal, and they have been compressed into their present condition in connection with that flattening out of the premandibular gut and the premandibular arches that has been caused by the descent of the infundibulum. From this primitive trabeculo-cranial snout what I have called the viscero-trabeculo-cranial one is developed by the growth forward of the bucco-pharyngeal upper lip to or beyond the tip of the pre-existing trabeculo-cranial snout, and its complete fusion with the latter, excepting in the Cyclostomata, along the median line. Associated with this forward growth of the lip, and to a certain extent caused directly by it, the nasal pits, the ethmoidal latero-sensory line, if present, and the point of attachment of the hypophysial cord or canal to the external ectoderm, are forced from the ventral to the dorsal surface of the snout, and as the point of attachment of the hypophysial canal or cord is the last one of the three to reach this position, its presence on the dorsal surface of the snout is definite indication that, at that stage of development, the snout was typically of the viscero-trabeculo-cranial type. It is, however, now known that in *Acipenser* the point of attachment of the hypophysial cord after being forced on to the dorsal surface of the snout shifts posteriorly on to its ventral surface (v. Kupffer, 1893; Holmgren, 1931), and it is probable that this takes place also in *Polypterus* and certain of the Teleostei, for in advanced embryos of the latter Fishes the canal or cord has its attachment on the dorsal surface of the buccal cavity, the ethmoidal latero-sensory line and the nasal apertures remaining on the dorsal surface of the snout. This type of snout may accordingly be called the modified viscero-trabeculo-cranial.

De Beer (1923, 1926), in connection with his works on the development of the hypophysis and the pituitary body, gives three diagrammatic figures which show the point of attachment of the hypophysial stalk, cord or canal in each of five large groups of vertebrates, but this apparently refers to the point of attachment of the stalk, cord or canal at the time of the greatest forward growth of the bucco-pharyngeal upper lip, although this is not so stated. These figures should accordingly indicate the type of the snout at that stage of development. According to these figures the hypophysis develops in connection with a Rathke's pocket in the Selachii and Amniota, and this pocket opens on the roof of the buccal cavity. The snout of these vertebrates should therefore be of the trabeculo-cranial type, and in accordance with this the nasal apertures and the ethmoidal latero-sensory line lie, in the Selachii, on its ventral surface. The Batoidei are doubtless included under the term Selachii, as employed by de Beer, and it is well known that in all of these Fishes the snout is of the trabeculo-cranial type. The Holocephali are not particularly mentioned, but as the nasal apertures of these Fishes lie definitely on the ventral surface of the snout, it is quite certain that the latter is of the trabeculo-cranial type, notwithstanding that what is apparently the homologue of the ethmoidal latero-sensory line of the Plagiostomi lies on its dorsal surface. The nasal apertures do not undergo dichotomous sub-

division in any of these Fishes, and this is peculiar to them of all Fishes excepting the Cyclostomata and, I believe, a few of the Teleostei.

In the Amphibia and Teleostomi the hypophysial stalk or cord is shown by de Beer attached to the ectoderm on the dorsal surface of the snout, which would indicate that the latter is of the viscero-trabeculo-cranial type. It is, however, known that in certain of the Teleostomi this point of attachment shifts, in later stages of development, from the dorsal on to the ventral surface of the snout, thus giving origin to what I have called the modified viscero-trabeculo-cranial type. The nasal apertures and the ethmoidal latero-sensory line, when present in these Fishes, always remain on the dorsal surface of the snout, and the nasal apertures always undergo dichotomous subdivision excepting possibly in a few of the Teleostei.

The Dipnoi are not particularly mentioned by de Beer, but because of the general shape of their snout and the position of the nasal apertures on its ventral surface, I have heretofore considered it to be of the trabeculo-cranial type. Watson (1926), however, says that the nasal apertures of these Fishes lay primarily on the dorsal surface of the snout and have shifted secondarily on to its ventral surface in connection with the reduction of the maxillaries. This, if correct, would suggest that the snout is of the modified viscero-trabeculo-cranial type, the regular dichotomous subdivision of the nasal apertures and the development of the hypophysis in relation to a solid stalk, as in the Holostei and Teleostei, being in favour of this view. The apparent absence of an ethmoidal prolongation of the main-infraorbital line in these Fishes would then be accounted for, for the shifting of the nasal apertures from the dorsal to the ventral surface of the snout, and the enclosure of the posterior nasal apertures in the buccal cavity, would have prevented its development.

In the Cyclostomata de Beer shows the naso-hypophysial canal opening on the dorsal surface of the snout, and this is correct for *Petromyzon*, the snout of that fish thus being typically of the viscero-trabeculo-cranial type. There is, however, question as to the snout of the Myxinoidae. In *Petromyzon*, when the bucco-pharyngeal upper lip in growing forward has reached the hind edge of the nasal pit, both it and the overlying trabeculo-cranial tissues grow forward together until the nasal pit has been swung forward and upward on to the dorsal surface of the snout, the bucco-pharyngeal upper lip then growing onward alone to form the tip of the snout. In the Myxinoidae the development of the snout is not definitely known and there is question as to the extent of the forward growth of the bucco-pharyngeal upper lip. The nasal pit apparently remains on the ventral surface of the snout and the snout grows forward beyond it there enclosing the nasal tube. But there is question as to whether this forward growth is entirely of trabeculo-cranial origin, as Stensiö (1927) apparently concludes, or partly of trabeculo-cranial and partly of visceral origin. The nasal tube in any event ends posterior to the tip of the snout, its external opening accordingly lying morphologically on its ventral and not on its dorsal surface, the snout thus apparently being a modified trabeculo-cranial

instead of a modified viscero-trabeculo-cranial one. When considering, in an earlier work (Allis, 1931*a*), v. Kupffer's (1900) descriptions of the early development of *Bdellostoma*, I considered one of his figures to indicate that the nasal tube of that fish was separated, near its anterior end, into two parts by a median vertical partition, but this is apparently incorrect, for so far as I can find from descriptions of this fish the nasal tube opens anteriorly by a single aperture, as it does in *Myxine*.

In fossil fishes it is often difficult to determine the character of the snout, for the point of attachment of the hypophysial cord or canal to the external ectoderm can of course not be determined. Furthermore, the position of the ethmoidal latero-sensory line can only be determined when the line has to a certain extent become enclosed in the dermal bones, and because of the crushing and distortion of the skull during fossilisation it is often impossible to determine whether the external nasal openings lay in the living fish on the dorsal or the ventral surface of the snout. The snout of *Cephalaspis* is, however, definitely of the viscero-trabeculo-cranial type, and that of *Pteraspis* quite certainly of the trabeculo-cranial, for that the snout of the latter fish is the homologue of that of *Myxine*, as Stensiö concludes, seems to me improbable.

In *Macropetalichthys* and *Epipetalichthys* (Stensiö, 1925) the external nasal openings lie on the ventral surface of the snout near its lateral edge, as they do in *Chlamydoselachus*. No ethmoidal latero-sensory line is indicated, but the snout is quite certainly of the trabeculo-cranial type, as it also is in *Pholidosteus*. In the other Arthrodira that I find described, the external nasal openings apparently lie sometimes on the ventral and sometimes on the dorsal surface of the snout, but it nevertheless seems probable that in all these fishes the snout was of the trabeculo-cranial type. The Heterostraci, Arthrodira and Elasmobranchii would therefore resemble each other in this respect as well as in the character of the latero-sensory lines.

In the fossil Crossopterygii the external nasal openings lie definitely on the dorsal surface of the snout and in certain specimens there are said to be two on each side of the head. The ethmoidal canal, when present, also lies on the dorsal surface of the snout. In *Diplocercides* (Stensiö, 1922*b*), as also in the recent *Polypterus*, the floor of the neurocranium is perforated by a canal which in an earlier work (Allis, 1931*b*) I considered to have given passage to a hypophysial cord, and because of this I then considered the snouts of these two fishes to be of the trabeculo-cranial type, but the conditions in *Acipenser*, above described, strongly suggest that, as in the latter fish, the snout of the Crossopterygii is of the modified viscero-trabeculo-cranial type. In the fossil Chondrostei it is probable that the snout was also of this same modified type, notwithstanding that the ethmoidal canal lies definitely on its ventral surface, for otherwise the external nasal openings would have had to have acquired their position on the dorsal surface of the snout wholly independent of any forward growth of the bucco-pharyngeal upper lip.

Comparing the conditions in *Ichthyostega* with those in the fishes above referred to, it seems quite certain that its snout is of the trabeculo-cranial type and that in this respect as well as in the character of its latero-sensory lines it is allied to the Arthrodira and Elasmobranchii rather than to either the Crossopterygii or the Dipnoi. Furthermore, it is probable that the external nasal apertures were undivided, as they are in the Elasmobranchii, and in an earlier work (Allis, 1919) I found the external nasal apertures of *Hypogeophis* so strikingly similar to those in *Chimaera* that I considered that these vertebrates had descended from a common ancestor.

LABYRINTHODONTIA

In these Stegocephali there are shallow grooves on the outer surfaces of certain of the cranial roofing bones and they are generally considered to have lodged membranous latero-sensory canals similar to those in the recent bony fishes, but Escher (1925) considers this improbable. According to him, the latero-sensory lines begin their development in the recent Amphibia as they do in the recent Fishes as strands of special tissue lying wholly in the ectoderm, but that the organs that arise in these strands may undergo repeated subdivision, and as the planes of subdivision may lie parallel to the line of the strand, or transversely or obliquely to it, groups of organs arranged according to various patterns may arise. In certain species, some of these organs sink below the ectoderm and so give origin to little sac-like pits which resemble the pits formed in the Plagiostomi in the early stages of development of the latero-sensory canals. There is, as in the Plagiostomi, no slightest indication of any infolding of the ectoderm, but the little pits do not here, as in the Plagiostomi, become connected with each other by strands of any kind, and none of them ever become enclosed in canals. No bones are developed in protective relation to these pits and there are even no depressions, grooves or other markings on the outer surfaces of the underlying bones to indicate the positions or even the presence of the pits. Comparing these conditions with those in the Labyrinthodontia, Escher concludes that there were in the latter no latero-sensory canals of any kind and to account for the latero-sensory grooves he suggests that lines of sac-like pits were developed and that they had associated with them numerous follicle-like mucous glands, and that the grooves were developed in relation to these lines much as the slight grooves that underlie the pit lines in certain of the recent Teleostomi are developed in relation to those lines. It seems to me much more probable, however, that latero-sensory open grooves similar to those in certain of the Elasmobranchii and Arthrodira were developed in these Stegocephali, and that they have shifted somewhat laterally and so given origin to the grooves actually found.

Moodie (1916), in his extensive work on the remains of the Stegocephali from the Coal Measure deposits of the United States, gives a figure, reproduced in the accompanying fig. 4, which is said to give in outline a dorsal view of the

skull and cranial bones of *Eryops*. On it he has traced what he considers to be the complete course of the latero-sensory grooves in these amphibians, but it is said to be a compilation of fragments of these grooves found on different specimens and species and not occurring on any single one. These grooves are said to be wide and shallow with smoothly rounded edges and along the bottom of certain of these grooves there is frequently a row of pits or depressions which are considered to have lodged the sense organs of the lines.

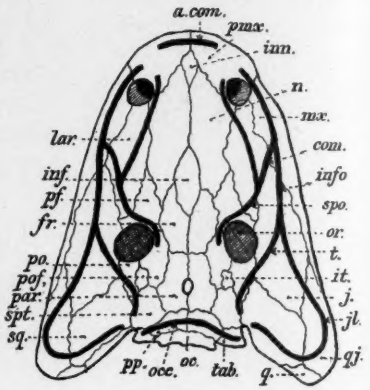

Fig. 4. Dorsal view of the skull of *Eryops* showing latero-sensory canals. After Moodie.

On the dorsal surface of the anterior end of the snout of *Eryops* there is on either side a so-called premaxillary bone which articulates in the median line with its fellow of the opposite side, and is prolonged latero-posteriorly to reach and form the antero-mesial boundary of the single external nasal opening of its side of the head, that opening lying wholly on the dorsal surface of the snout. On the dorsal surface of this bone there is a short ethmoidal cross-commissural groove which is directly continuous in the median line with its fellow of the opposite side, and lateral and mesial, respectively, to the nasal opening the anterior ends of the infraorbital and supraorbital grooves extend a short distance on to the dorsal surface of the bone. If latero-sensory ossicles were here developed in relation to these several grooves, the bone would accordingly contain rostral, dorsorostral and nasal components and also a component corresponding to the anterior portion of the antorbital bone of *Amia* and *Polypterus*. It seems much more probable, however, that the bone is wholly of membrane origin and corresponds to one half of the anterior portion of the rostro-interrostral of *Ichthyostega* completely fused with the rostro-premaxillary, but with the premaxillary portion of the latter bone reduced to a narrow band and swung forward and upward to such an extent that the ethmoidal canal lies on the dorsal instead of on the ventral surface of the snout.

Posterior to the two premaxillaries (dorsorostro-premaxillaries) and wedged in between their hind ends and the anterior ends of the so-called nasals there is a small median lenticular-shaped bone called by Moodie the internasal and also so called by Gregory (1920) in his reproduction of this figure. It evidently corresponds to the posterior median dorsorostral (pineal plate) of *Coccosteus* and apparently also to the posterior portion of the rostro-interrostral (median dorsorostral) of *Ichthyostega*.

The so-called nasal of either side articulates with that part of the hind edge of the premaxillary (dorsorostro-premaxillary) that lies between the

median dorsorostral and the dorso-mesial edge of the nasal opening, and it extends posteriorly from there nearly to the level of the anterior edge of the orbital opening. Mesially it articulates with the internasal (median dorsorostral), posterior to that with its fellow of the opposite side, and then with the anterior portion of the lateral edge of a small lenticular-shaped bone that is wedged in between the hind ends of the nasals of opposite sides and is called by both Moodie and Gregory the interfrontal. Posteriorly the nasal articulates with the anterior end of the so-called frontal which extends forward along the lateral edge of the so-called interfrontal, between it and the hind end of the nasal. Anteriorly, at the widest part of the bone, its lateral edge forms the mesial boundary of the external nasal opening, and posterior to that articulates laterally first with a so-called lachrymal and then with a so-called prefrontal, the latter two bones completely filling the space between the nasal and orbital openings and articulating ventro-laterally with the so-called jugal and maxillary. Dorso-mesial to the orbital opening the prefrontal articulates with the anterior edge of a so-called postfrontal which forms the larger part of the dorso-mesial boundary of the orbital opening and extends posteriorly a certain distance beyond it. The so-called nasal thus corresponds in topographical position to an inner lateral dorsorostral of Fishes fused with a small outer lateral dorsorostral and is apparently the equivalent of the so-called naso-postrostral of the Ichthyostegalia fused with the small so-called anterior ant-orbital. The so-called lachrymal and prefrontal would then correspond, respectively, to the anterior and posterior preorbitals of my descriptions of Fishes.

The supraorbital groove which begins on the dorsal surface of the premaxillary (dorsorostro-premaxillary) runs posteriorly across that part of the nasal (posterior dorsorostral) that forms the dorso-mesial boundary of the nasal opening and then across the lachrymal (anterior preorbital) and prefrontal (posterior preorbital) to the dorso-posterior corner of the latter bone where it reaches the so-called postfrontal and extends posteriorly nearly to the middle of the length of that bone. Between the nasal and orbital openings the groove makes a deep lateral bend at the summit of which it is connected by a communicating branch with the main-infraorbital groove as that groove traverses the maxillary. The supraorbital latero-sensory groove thus here lies throughout its entire length on the outer surfaces of a line of bones that, excepting in their relations to this latero-sensory line, correspond strictly to certain of the bones of the outer lateral row of dorsocranials of the Ichthyostegalia and the non-Arthrodiran Osteichthyis. The groove has certainly been developed in some relation to an overlying latero-sensory line, and this line was probably enclosed in an open groove and not in a closed canal, as already fully explained. The canal of the Ichthyostegalia lies close to the lateral edges of the frontal and fronto-parietal, and probably equally close to the lateral edge of the inner lateral dorsorostral component of the so-called naso-postrostral. A slight lateral shifting of the line would accordingly give it the relation to the outer

lateral row of cranial membrane bones that it actually has in the Labyrinthodontia, and if the line had persisted as an open groove the conditions in the Labyrinthodontia would have arisen. In the Coelacanthidae, it is to be noted that a considerable part of the supraorbital line lies in a groove between the frontal and the supraorbitals, and there is question as to its having been there enclosed in bone of any kind.

The frontal (anterior frontal) is a small bone the anterior end of which fits in between the interfrontal (posterior median dorsorostral) and the hind end of the nasal (posterior dorsorostral). Mesially it articulates with its fellow of the opposite side, laterally with the prefrontal (posterior preorbital) and the so-called postfrontal and posteriorly by a nearly straight transverse hind edge with the anterior edge of the parietal (posterior frontal). The bone is not traversed by any of the latero-sensory grooves.

The parietal (posterior frontal) articulates in the median line with its fellow of the opposite side, laterally with the so-called postfrontal and a so-called supratemporal and posteriorly with a bone called by Moodie the postparietal, but by Gregory the dermo-supraoccipital. The parietals of opposite sides enclose between themselves the parietal foramen and they are not traversed by any of the latero-sensory grooves.

The so-called postparietal articulates in the median line with its fellow of the opposite side and laterally both with a small so-called tabular and the so-called supratemporal. Posterior to the postparietals of opposite sides there is a small median bone called by Moodie the occipital. Each of the postparietals is traversed transversely its full length by the supratemporal latero-sensory groove and corresponds topographically to the parieto-extrascapular of the Ichthyostegalia and the similarly named bone in Fishes. The small so-called tabular which lies lateral to the posterior portion of this bone apparently corresponds to the membrane component of the lateral extrascapular of Fishes and hence forms the posterior one of the line of outer lateral membrane bones. It is shown traversed on one side of the head, but not on the other, by the supratemporal latero-sensory groove, and it is not traversed on either side by the main-infraorbital groove, that groove beginning near the hind edge of the so-called supratemporal.

The so-called supratemporal forms the lateral boundary of this part of the roofing bones of the skull, articulates mesially with the postparietal (parieto-extrascapular) and with the lateral edge of the posterior portion of the parietal (posterior frontal) and anteriorly with two bones called by both Moodie and Gregory the intertemporal and postfrontal. The so-called intertemporal is a small somewhat rectangular bone which articulates mesially and antero-mesially with the so-called postfrontal and laterally and antero-laterally with a so-called postorbital, the latter bone and the so-called postfrontal articulating with each other anterior to the intertemporal and together forming the dorsal and dorso-posterior boundaries of the orbital opening. The main-infraorbital groove begins on the so-called supratemporal near its hind end and not far

from its lateral edge, and running forward in that position nearly to the anterior end of the bone turns antero-laterally posterior to the so-called intertemporal and traverses the so-called postorbital. The so-called supratemporal thus evidently corresponds to the pterotic portion of the outer lateral dorsocranial of *Polyodon*, the intertemporal which is not traversed by any portion of either of the latero-sensory grooves corresponding to part of the sphenotic portion of that bone, the postfrontal corresponding to the supraorbital and part of the sphenotic portion of the same bone of *Polyodon*, and the postorbital corresponding to the membrane component of the suprapostorbital together with that part of the sphenotic portion of the bone of *Polyodon* that I have called the spiracular process, thus apparently corresponding to the supra-postorbito-membranosphenotic of Fishes.

The main-infraorbital groove, after having traversed the postorbital (supra-postorbito-membranosphenotic), reaches and traverses a long bone called by both Moodie and Gregory the jugal, which forms the ventral border of the orbital opening and extends forward about half-way between that opening and the nasal opening, its pointed anterior end being wedged in between the lachrymal (anterior preorbital) and a bone called by Moodie the maxillary but by Gregory the jugal. The latter bone extends the full length of the lateral edge of the skull, its anterior portion forming the ventro-lateral and part of the posterior boundary of the external nasal opening. The infraorbital groove, after traversing the bone called by Moodie the jugal, passes on to the maxillary and running forward to its anterior edge extends slightly on to the dorsal surface of the premaxillary.

Part of the premaxillary, the mesial portion of the nasal, the frontal, the parietal and the parietal portion of the interparietal (parieto-extrascapular) thus quite certainly correspond to the inner lateral row of membrane bones of Fishes, the extrascapular component of the parieto-extrascapular apparently corresponding to the membrane component of the mesial extrascapular of Fishes. The lateral part of the premaxillary, the lateral portion of the nasal, the lachrymal, prefrontal, postfrontal, postorbital, intertemporal, supra-temporal and lateral extrascapular would then correspond to the outer lateral row of membrane bones of Fishes, and it is to be noted that several of the bones of this latter row have to the bones of the inner lateral row the topographical relations that the scales of a longitudinal row on the trunk of Fishes have to the scales of the adjacent rows. The so-called lachrymal of these amphibians is thus certainly not the homologue of the similarly named bone of Fishes, for that bone is developed in relation to the main-infraorbital latero-sensory line and is here apparently represented in the anterior portion of the jugal.

In the other Labyrinthodontia described and figured by Moodie there are slight differences in the arrangement of the bones and the course of the latero-sensory grooves, but the general principle of arrangement is the same as in *Eryops*. In the four figures shown by him in the plate accompanying his 1908 work, the postfrontal (supraorbito-membranosphenotic), intertemporal

(membranosphenotic) and postorbital (suprapostorbito-membranosphenotic) are replaced by two bones called the postfrontal and postorbital, and in *Anaschisma browni* the hind end of the supraorbital groove meets and anastomoses with the main-infraorbital in the postorbital. In this same work Moodie gives a diagrammatic figure said to show the general course of the latero-sensory "canals" in the Stegocephali, but the course of the canals (grooves) and the related bones differ markedly from those in *Eryops* as just above described. In it the membranosphenotic has apparently fused with the frontal (anterior frontal) and is traversed by the main-infraorbital groove, the nasal (posterior dorsorostral) has been prolonged posteriorly to such an extent that it forms the greater part of the dorsal margin of the orbital opening and is traversed by the supraorbital groove. The so-called lachrymal corresponds in position to what I have called in *Eryops* the posterior preorbital and the anterior preorbital has apparently fused completely with the maxillary, the anterior portion of the latter bone accordingly being traversed by both the main-infraorbital and supraorbital grooves.

The condition of the latero-sensory lines of the Labyrinthodontia thus indicates either reversion from that in the Ichthyostegalia or descent independently of the latter Amphibians from a common ancestor related to the Arthrodira and possibly also to the earliest Crossopterygii, and the character of the snout is in favour of the latter assumption.

The external nasal openings and the ethmoidal latero-sensory grooves lie on the dorsal surface of the snout. The snout is accordingly of the viscero-trabeculo-cranial type, but there seems nothing to indicate definitely whether it is of the typical or modified type. The snout of the recent Amphibia is also of the viscero-trabeculo-cranial type and probably a typical one, for de Beer, in his diagrammatic figures, shows the hypophysial cord attached to the ectoderm on its dorsal surface. The snout of the Labyrinthodontia and also that of the recent Amphibia thus differs markedly from that of the Ichthyostegalia, but could have been readily derived from it by the growth forward of the bucco-pharyngeal upper lip in early embryonic stages. It seems more probable, however, as just above stated, that these Amphibia and the Ichthyostegalia represent two distinctly different lines of descent from an early common ancestor. There are in the Labyrinthodontia, as in the Ichthyostegalia, the recent Amphibia and certain of the recent Elasmobranchii, internal nasal openings, and similar openings have been described in certain of the early Crossopterygii. In the latter Fishes, as in the Labyrinthodontia, the main-infraorbital canal was not continuous with the ethmoidal line.

SUMMARY AND CONCLUSIONS

The latero-sensory system is of very ancient origin, the earliest known vertebrates having possessed it, and from that time down to the present the organs of the several principal and well-known lines have persisted in all

Vertebrates that have lived uninterruptedly in the water with probably but slight if any change in character or function. These organs lay primarily in the ectoderm, exposed on its outer surface, but being sensitive structures it was of advantage to have them protected as much as possible from injury, and so far as can be judged from what is actually known about them, this has been accomplished in two distinctly different ways, each way apparently being definitely characteristic of a large group of the possessors of these organs. In one of these groups, which includes the Heterostraci (*Pteraspis*), Arthrodira, Elasmobranchii and Amphibia, certain organs of the lines, or the entire lines, apparently became detached from the ectoderm in which they primarily lay and sank beneath it, thus giving origin to sac-like pits, to open grooves or closed canals, while in the other group which includes the Osteostraci (*Cephalaspis*), Cyclostomata, Crossopterygii, Dipnoi and Actinopterygii, the individual organs of a line became enclosed in short grooves or canals formed by the infolding of the entire ectoderm, the so-formed canals fusing with each other in certain Fishes to form continuous closed canals. When a canal was formed by an infolding of the entire ectoderm, there was normally but a single primary pore or tube between each two adjacent organs of a line, and independent latero-sensory bones were usually developed in protective relation to the canals, but when the latero-sensory lines became detached from the ectoderm and sank inward beneath it preliminary to the formation of canals, two or more primary pores or tubes might be formed between two successive organs of a line, and no independent bones developed in protective relation to the organs can be recognised.

In the Fishes of the first group, the primarily single external nasal aperture of either side apparently never undergoes complete subdivision into two, while in the Fishes of the second group, with the exception of the Cyclostomata, this aperture almost invariably undergoes dichotomous subdivision.

In the recent Amphibia and in certain of the recent Elasmobranchii there are internal nasal apertures that are considered to be the homologues of the choanae of higher Vertebrates, and as the Devonian Amphibia possessed these apertures, it seems reasonable to assume that the Devonian ancestors of certain of the Elasmobranchii also possessed them. Internal nasal apertures considered to be the homologues of those of the Amphibia have also been described in certain of the Devonian Crossopterygii, but they have not been described in any other Fishes of the second group, either fossil or recent, excepting only the Dipnoi, and in these latter Fishes the internal nasal apertures are quite certainly not the homologues of those in the Amphibia and Elasmobranchii. The possession of these apertures must evidently have been of some advantage for they were apparently acquired independently and at about the same geological time in certain of the Elasmobranchii, Amphibia, Dipnoi and Crossopterygii, and excepting in the latter Fishes they have apparently been retained uninterruptedly down to the present time. It must therefore be that these early Crossopterygii either had no descendants, that those descendants did not

inherit the internal nasal apertures, or that they gave origin to some branch of the Amphibia.

It therefore seems quite certain, if my interpretation of the latero-sensory canals is correct, that the Ichthyostegalia have descended from an ancestor related to the Arthrodira-Elasmobranchian line and that the other Amphibia are either descendants of the Ichthyostegalia, as Säve-Söderbergh concludes, or have descended independently from an ancestor related both to the Arthrodira and to those of the early Crossopterygii that possessed internal nasal apertures. The Dipnoi would belong to a wholly different line.

The pattern of arrangement of the cranial roofing bones of the Amphibia presents the five longitudinal rows of membrane bones so characteristic of the Teleostomi, but there are apparently no latero-sensory bones. The lateral and mesial extrascapulars probably correspond to the membrane components alone of those bones of Fishes. There is no nasal bone comparable to that in Fishes. The so-called lachrymal is one of the outer lateral row of membrane roofing bones and accordingly is not the homologue of the lachrymal of Fishes, for the latter bone is developed in relation to the main-infraorbital latero-sensory line.

A Rathke's pocket has been described only in Vertebrates in which the snout is of the trabeculo-cranial type, that is in the Amniota, Plagiostomi and Myxinoidae, and in the latter Fishes it is developed in a manner markedly different from that in the Plagiostomi. When the snout is of the viscero-trabeculo-cranial type, that is in the recent Petromyzontidae, Crossopterygii, Dipnoi, Actinopterygii and Amphibia, the pocket is replaced by what is generally considered to be a solid ingrowth of the ectoderm, but this solid ingrowth is probably simply a fold or pocket of the ectoderm the walls of which have been so closely pressed together that the cavity of the fold or pocket has been entirely suppressed. The presence or absence of the pocket thus seems to be definitely associated with the extent of forward growth of the bucco-pharyngeal upper lip. In the Ichthyostegalia, where the snout is quite certainly of the trabeculo-cranial type, it is therefore quite probable that there was a Rathke's pocket. In all of the other Amphibia, both recent and fossil, the snout is apparently of the viscero-trabeculo-cranial type. It is therefore probable that descendants of these latter Amphibia could have acquired a Rathke's pocket only in connection with reversal from a viscero-trabeculo-cranial snout to a trabeculo-cranial one.

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EXPLANATION OF LETTERING

A.	Anterior antorbital.
a.com.	Anterior commissure of lateral line canals.
Ant.	Posterior antorbital.
apv.	Anterior palatal vacuity.
c.a.	Probable course of anterior commissure of the infraorbital canals.
com.	Commissural communication between infraorbital and supraorbital canals.
Dpl.	Dermopalatine.

Enpt.	Entopterygoid.
Enptq.	Quadrate ramus of entopterygoid.
Ext.l.	Lateral extrascapular.
Ext.m.	Parieto-extrascapular.
Eye.	Orbital opening.
Fr., fr.	Frontal.
inf.	Interfrontal.
info.	Infraorbital lateral line canal.
inn.	Internasal.
it.	Intertemporal.
Ju., j.	Jugal.
juc., jl.	Jugal canal.
La., lar.	Lachrymal.
lc.	Cephalic division of main lateral line canal.
mx.	Maxillary.
n.	Nasal.
Na.ptr.	Naso-postrostral.
n.e.	External nares.
n.i.	Internal nares.
oc.	Occiput.
occ.	Occipital cross-commissure of the lateral line system.
or.	Orbit.
ot.	Otic part of the infraorbital canal.
Pa.	Fronto-parietal.
par.	Parietal.
pf.	Prefrontal.
pmx.	Premaxilla.
po.	Postorbital.
Po.dsph.	Postorbito-dermosphenotic.
poc.	Preopercular canal.
pof.	Postfrontal.
Pop.	Preopercular.
pp.	Postparietal.
ptc.	Postotic canal.
q.	Quadrate.
qc.	Quadrato-jugal canal.
qj., Quj.	Quadrato-jugal.
R.ir.	Rostro-interrostral.
R.pmx.	Rostro-premaxillary.
R.pmxc.	Rostral part of the rostro-premaxillary.
R.pmxd.	Tooth-bearing part of the rostro-premaxillary.
so.	Suborbital part of the infraorbital canal.
So_1.	Anterior supraorbital.
So_2.	Posterior supraorbital.
soc., spo.	Supraorbital lateral line canal.
spt.	Supratemporal.
Sq., sq.	Squamosal.
St.it.	Supratemporo-intertemporal.
t.	Temporal lateral line canal.
tab.	Tabular.
Vo.	Vomer.

ANATOMICAL NOTES

THE STERNALIS MUSCLE IN THE ANENCEPHALOUS FOETUS

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Six anencephalous foetuses were dissected and the sternalis muscle was found in three.

(1) *Full-time female foetus, anencephalous with spina bifida in lower dorsal and lumbar regions. Sternalis unilateral, right.* Fig. 1.

The muscle arose by fleshy fibres from the fascia over the lower part of the manubrium and upper part of the body of the sternum and from the third right costal cartilage. In this situation its fibres were firmly blended with those of the pectoralis major. The muscle passed downwards and outwards to be inserted mainly into the aponeurosis of the external oblique. The distal part of the muscle had no direct connection with the pectoralis major which lay deeply to it, but a few of its fibres passed on to the deep fascia over the attachment of that muscle to the sixth costal cartilage. The length was 3·5 cm. and the maximum breadth 1 cm. The upper

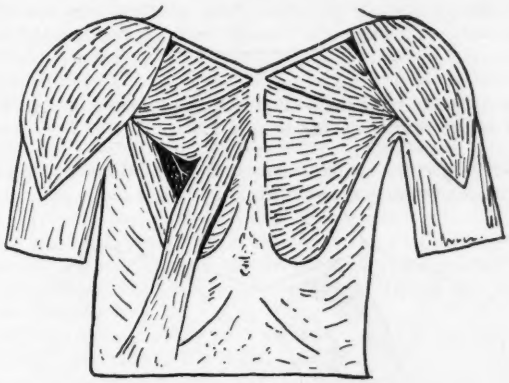

Fig. 1.

part of the pectoralis major of this side was well developed, but its lower portion was greatly thinned particularly towards the sterno-costal attachment. A triangular gap was present between these two portions. This gap was filled with loose areolar tissue and partly covered by the intermediate portion of the sternalis.

The left pectoralis major and the platysma were well developed.

The nerve supply was derived from the medial anterior thoracic. This nerve pierced the pectoralis minor, crossed the gap in the pectoralis major and entered the outer border of the sternalis. Terminal twigs of the third intercostal nerve pierced the muscle on their way to the skin.

(2) *Full-time female foetus, anencephalous with spina bifida in lumbar region. Sternalis bilateral.* Fig. 2.

The condition here was almost bilaterally symmetrical. The origin of each muscle was from the upper part of the body of the sternum and from the second and third costal cartilages. In this situation there was firm union with the fibres of the pectoralis major. The direction of the fibres in both was downwards and outwards to an insertion

on the fifth costal cartilage and the adjacent part of the fifth rib. A well-marked triangular gap was present in the intermediate part of each pectoralis major, but in contrast to case 1 the fibres of insertion of each sternalis lay deep to those of the lower portion of that muscle. The length was approximately 3 cm. and the maximum breadth 1·2 cm. in each case. There was no connection with the platysma or sterno-mastoid.

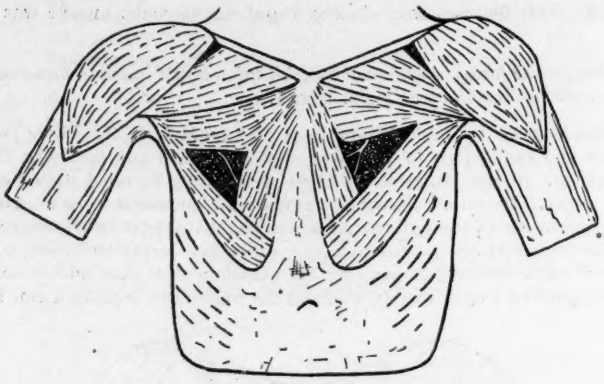

Fig. 2.

The nerve supply was from the medial anterior thoracic in both and reached the muscles after a course similar to that in case 1.

(3) *Full-time female foetus, anencephalous with spina bifida in cervical and thoracic regions. Sternalis unilateral, right.* Fig. 3.

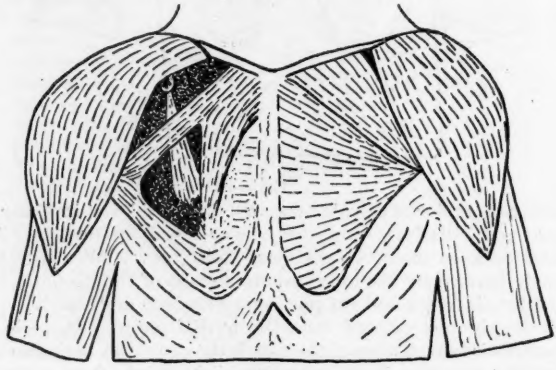

Fig. 3.

The sternalis in this case was not of large size. It arose by fleshy fibres from the fascia over the upper part of the manubrium and from the right sterno-clavicular joint. In this region it was firmly fused to the pectoralis major. From this attachment the muscle passed downwards almost vertically, narrowing as it descended to an insertion on the fourth costal cartilage. The pectoralis major of this side exhibited a large triangular deficiency which was only partly covered by the lower fibres of the sternalis. In the gap the fibres of origin of the pectoralis minor could be seen.

The clavicular origin of the pectoralis major of the same side was limited to a small area at its medial end, while its lower portion arose from the sixth and seventh costal cartilages. Near the insertion of the muscle into the humerus the two portions joined almost at right angles. The deltoid was normal and well developed, but owing to the small clavicular attachment of the pectoralis major an appreciable interval, in which the tip of the coracoid process could be seen, was present between the adjacent borders of these muscles.

Both the lateral and medial anterior thoracic nerves were observed entering the pectoralis major but no distinct twig was traced to this abnormal muscular slip.

The term "sternalis muscle" has been employed in these descriptions, as the examples resemble those similarly named by Shepherd (8, 9) and others. The appearance of the parts and the innervation of the muscles, however, clearly indicate that they have been formed by a deviation or dislocation of some of the fibres of the pectoralis major.

The sternalis has been variously described as a vestige of the panniculus adiposus, a homologue of the sterno-cleido-mastoid, a portion of the pectoralis major, and as an extension upwards of the rectus abdominis. These views have been discussed by Turner (11), who considered that the evidence was in favour of classifying it with the platysma. Some evidence in favour of regarding certain cases as derivatives of the pectoralis major has been adduced, particularly by Bardeleben, Shepherd, Cunningham (3, 4), Windle, and Abraham. Bardeleben (2) was the first to enunciate this view, but as he observed twelve cases in adults all innervated by intercostals he restricted those derived from this source to 21 per cent. Shepherd (8, 9) traced the nerve supply to ten sternalis muscles occurring in anencephalous foetuses; all were supplied by the anterior thoracics, but two also received an additional supply from intercostals. In every case where the pectoralis major was deficient the nerve supply was derived from the anterior thoracics alone. In the two cases which received an additional supply from the intercostals there was no such deficiency. This led him to consider that there were probably two different kinds of sternalis.

As indicating the relationship of the muscle to the pectoralis major, Windle (12) and Abraham (1) also stressed the fact that in the anencephalous foetus in particular, the presence of the muscle was frequently associated with a thinning or an actual gap in the pectoralis major.

Hallet (6), in 1848, seems to have been the first to record the nerve supply to the sternalis in an adult; it was from the third, fourth, and fifth intercostals. Dwight (5) recorded five cases occurring in adults. Of these, two were innervated by intercostals, one by one of the ? anterior thoracic nerves, while in two it could not be made out. The same author also described a bilateral sternalis associated with a definite interspace in both large pectorals but did not give its nerve supply.

Cunningham (4), in 1888, tabulated the source of the nerve supply in cases recorded up to that date, and found that in thirty-three it was derived from the anterior thoracics and in five from the intercostals.

Smith (10) recorded a bilateral sternalis in an adult supplied by the second and third intercostals and was certain that no twigs entered it from the thoracics. Recently, I observed a bilateral sternalis in an adult dissecting room subject which agreed in size, position and innervation with that described by Smith (10). It was entirely superficial to well-developed pectoral muscles and deep to the platysma.

Patten (7) recently described two cases of right sternalis in adult dissecting room subjects both innervated by the external anterior thoracic nerve.

No satisfactory explanation can be advanced as to the remarkable frequency with which this abnormality occurs in the anencephalous foetus. The fact that its nerve supply may be derived from one or other of the anterior thoracics shows that the same set of fibres is not always implicated in its formation. Further, the varia-

bility of its nerve supply, particularly in the adult, appears to indicate that the muscle is not constantly derived from the same source.

Summary

Three examples of sternalis muscle in the anencephalous foetus are described. The appearance of the parts and the innervation of the muscles indicate that they have been derived from the pectoralis major.

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UNUSUAL COURSE OF VENA HEMIAZYGOS

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Among the numerous irregularities of the azygos veins which have been described, the following as far as I have been able to ascertain has not been mentioned. The azygos veins were regular with the exception that the vena hemiazygos instead of passing dorsal to the thoracic aorta took a course ventral to this vessel to join the vena azygos. This position of the hemiazygos vein ventral to the aorta may be explained by a persistence of the smaller ventral portion of the pre-vertebral plexus with an obliteration of the larger posterior portion.

REVIEWS

Topographical Anatomy of the Dog. (3rd ed.) By O. CHARNOCK BRADLEY, M.D., D.Sc., F.R.C.V.S. (Edinburgh: Oliver and Boyd, Ltd.) 1935. Pp. 284, figs. 91. Price 25*s.* net.

This distinguished practical manual of the dissection of the dog is now in its third edition. The text is lucid, adequate and direct. The instructions for dissection are simple, clear and convenient. The illustrations, which number 91, are excellent, and many are in colour. Anyone—veterinary surgeon, laboratory worker, comparative anatomist—who needs to know or orientate himself in the anatomy of the dog, has in Professor Bradley's book the most complete and handiest guide he could wish for.

The Microscopic Anatomy of Vertebrates. By G. G. SCOTT, Ph.D., and J. I. KENDALL, Ph.D. (London: Henry Kimpton.) 1935. Pp. 306, figs. 167. Price 17*s.* 6*d.* net.

The authors have prepared this text-book as a companion to those which deal with the gross anatomy and embryology in courses of vertebrate anatomy. The book consists of 306 pages including 167 illustrations, lists of additional reading, an appendix of technical instructions, a list of laboratory preparations and an index. The book is very well done and should be most useful.

Gray's Anatomy, Descriptive and Applied. (26th ed.) Edited by T. B. JOHNSTON, M.B., Ch.B. (London: Longmans, Green & Co. Ltd.) 1935. Pp. xxxii + 1516, figs. 1323 (611 in colour). Price 42*s.* net.

We heartily congratulate Professor Johnston upon the excellence of this new edition of a familiar authoritative textbook, which constitutes an eloquent tribute to his ripe experience, painstaking labour and wisdom of judgment. Striking improvements have resulted from the enlargement and re-writing of many sections, by the liberal addition of new and superior illustrations, by the inclusion of the most recent findings of anatomical research, and by the mode of treatment of those subjects difficult of student comprehension (e.g. embryology, peritoneum, central and sympathetic nervous systems).

An attractive and lucid style facilitates reading and a most praiseworthy sense of balance and proportion is maintained throughout.

The book remains essentially practical as ever; a reliable guide to the student and a proven friend to the post-graduate.

Minor criticisms affect mainly points which permit some diversity of opinion—e.g. the subclavian artery still grooves the first rib and the insertion of omohyoid might be more conventional. A reference to Negus's laryngological researches might be justly included.

We warmly recommend this work and again compliment the editor.

THE EFFECTS OF OESTRONE ON THE PROSTATE AND UTERUS MASCULINUS OF VARIOUS SPECIES OF PRIMATE

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AND A. S. PARKES

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It is known that rudimentary structures in the male monkey, generally believed to be derived from the Müllerian ducts, respond to oestrogenic substances in a manner characteristic of functional organs of the female with a similar embryological origin (see Parkes and Zuckerman, 1935, for references). One such rudiment is the uterus masculinus. In the usual laboratory Primate, the rhesus macaque, this structure is clearly vaginal in histological character; it responds to oestrone by epithelial growth and cornification in the same way as does the vagina of the female. Comparative study has shown that in certain other Primate types the uterus masculinus is "uterine" in histological form, and by analogy with the functional uterus of the female, a "uterine" uterus masculinus might be expected to respond to prolonged oestrin treatment by glandular hyperplasia (see Zuckerman and Parkes, 1935). The possibility of glandular hyperplasia of the uterus masculinus being concerned in the aetiology of glandular enlargement of the prostate in Man has been considered in our previous studies, and the general thesis that Müllerian tissues are concerned in this condition receives some support, from a different angle, in the observations of Burrows (1935).

Our present paper is mainly devoted to oestrone-induced changes in the uterus masculinus of various species of Primate; other similarly induced changes in the prostate have also been investigated, in view of the possibility that they too may throw light on the clinical condition of prostatic enlargement.

MATERIAL

In both species of monkey which formed the subject of our previous report (*Macaca mulatta*, the rhesus monkey, and *Papio papio*, the Guinea baboon), the uterus masculinus responds to oestrone injections by stratified epithelial growth and cornification. The Barbary ape, *Macaca inuus*, reacts in the same way (Courrier and Gros, 1935). Table I gives a list of six new species which we have investigated, mainly in the hope that in some of them the uterus masculinus would prove to be glandular or uterine in type. The list is unavoidably short, for it is only rarely that dealers have for sale examples of any but a few common species of monkey.

The normal condition of the uterus masculinus in the marmoset, the common macaque, and the mona monkey has already been described (Zuckerman

and Parkes, 1935). A second specimen of the common macaque (MM. 58), a further specimen of marmoset (MM. 71) and a green monkey (MM. 61) formed additional control material for the present experimental series. For information regarding the normal uterus masculinus of the capuchin and langur we have referred to Heller and Sprinz (1921).

EXPERIMENTAL RESULTS

Since the publication of our earlier report we have found that the uterus masculinus of the rhesus monkey will respond to much smaller amounts of oestrone than were used in our first experiments. Doses of as little as 100γ daily for 14 days will regularly cause extensive stratification of the utricular epithelium of immature rhesus monkeys; for this reason the dosage of oestrone was reduced to that used in the present experiments. There were no external effects in the animals investigated, such as occur during oestrone injections into the male hamadryas baboon (Dohrn and co-workers, 1933), the male rhesus monkey (Bachman and co-workers, 1935; Parkes and Zuckerman, 1935) and the male Barbary ape (Courrier and Gros, 1935), except in the case of the common macaque. In this animal they comprised only turgid swelling of the anus.

Table I. *The effect of oestrone on the uterus masculinus in various species of monkeys*

Number of animal	Species	Weight (gm.)	Amount of oestrone daily (γ)	Days injected	Condition of uterus masculinus
MM. 69	Common marmoset (*Hapale jacchus*)	130	100	22	No obvious change
MM. 29	Brown capuchin (*Cebus fatuellus*)	1800	200	17	,,
MM. 59	Common macaque (*Macaca irus*)	1180	100	14	Extensive stratification
MM. 28	Green monkey (*Cercopithecus aethiops sabaeus*)	2200	200	14	,,
MM. 35	Mona Monkey (*Cercopithecus mona*)	1600	200	16	,,
MM. 56	Hanuman langur (*Presbytis entellus*)	4060	200	39	Glandular hyperplasia

Hapale jacchus: *the common marmoset*, MM. 69

Oestrone appears to have had no effect on either the uterus masculinus or the prostatic glands of this animal. The utriculus has a simpler form than that in either the control specimen previously described (Zuckerman and Parkes, 1935), or in the additional control we obtained, comprising as it does a main central lumen with simple glandular offshoots. In total length it measures 0·56 mm. Corresponding dimensions in the two controls are 0·6 and 0·7 mm. respectively. The utricular epithelium in the injected specimen shows no sign of abnormal growth or stratification, and, as in the normal animal, is typically composed of one or two layers of cuboidal or low columnar epithelium. Some epithelial growth appears to have taken place in part of the prostatic urethra; the rest of the prostate presents no abnormal characters.

Cebus fatuellus: *capuchin*, MM. 29

According to the description and figures provided by Heller and Sprinz (1921) the normal condition of the prostate and uterus masculinus in this species of Primate closely resembles that of the marmoset (Zuckerman and Parkes, 1935).

The experimental animal of the present series presents no obvious characteristic which can be attributed to the injection of oestrone. There is no hyperplasia of the small uterus masculinus, and, indeed, the only outstanding finding is the presence of many small tubular glands within the thickened epithelium of the crista urethralis.

Macaca irus: *common macaque*, MM. 59

As in the normal specimen of this species which we have already described, our second control prostate (MM. 58) is almost completely identical with that of the normal rhesus monkey. The maximum transverse span of the T-shaped uterus masculinus of the second control specimen is 0·5 mm., the maximum diameter of the whole prostate being 4 mm.

The uterus masculinus of the oestrone-injected common macaque shows considerable expansion and cornification, and is the usual flask-like diverticulum from the urethra also found in the rhesus monkey after similar treatment with oestrone. Mitotic figures are frequent in the deeply-stained basal layers of the extensively stratified epithelium, and by its expansion the uterus masculinus has compressed its stroma against the fibro-muscular capsule within which it lies. The maximum diameter of the uterus masculinus is about 2 mm., as compared with an average diameter for the whole prostate of 8 mm.

The fibro-muscular tissue of the prostate seems more abundant in relation to the number of glands than in the control specimens. The prostatic glands and the common ejaculatory ducts do not show any changes.

Cercopithecus mona: *mona monkey*, MM. 35

The most pronounced prostatic change in this animal is the extensive hyperplasia of the uterus masculinus, which appears as a flask-like diverticulum similar to that of the common macaque just described. At its widest part the uterus masculinus is a solid epithelial plate 2·1 mm. in diameter. The utricular stroma forms a narrow band of polygonal cells within the usual fibro-muscular capsule.

The stratified epithelial response of the uterus masculinus of the mona monkey is noteworthy in view of the fact that in the normal uninjected animal the uterus masculinus has several glandular offshoots which, like the main lumen, are lined by a pseudo-stratified columnar epithelium.

Other significant changes in the prostate of the mona monkey include extensive stratification of the epithelium of the dorsal part of the lower half of the prostatic urethra, as well as marked hyperplasia of the epithelium of the

lowermost parts of the common ejaculatory ducts. These are widely dilated, and are lined by four to six layers of large epithelial cells, many of which form peninsular extensions into the main lumen, into which, too, many epithelial cells are being shed. There are also pseudo-glandular extensions of the epithelium into the stroma surrounding the ducts.

There are relatively few glands in the body of the prostate itself, which consists mainly of fibro-muscular tissue. Many mitotic figures in the epithelium of the prostatic glands give evidence of growth, and in places the columnar cells are heaped. Small intraglandular papillary processes are also frequently encountered.

Cercopithecus aethiops sabaeus: *green monkey*, MM. 28

The maximum dimensions of the fixed prostate of our normal control green monkey, which was approximately the same weight and age as the injected animal, were 7·5 mm. from side to side, and 3 mm. dorso-ventrally (excluding the urethra). The uterus masculinus has the typical T-shape. Its very narrow lumen contains some secretion, and is bounded by a cuboidal epithelium which is typically one to two layers deep. The maximum transverse span of the limbs of the uterus masculinus is 1·3 mm. The common ejaculatory ducts are distended, and are lined by simple epithelium one to two layers deep, as are also the prostatic glands.

Corresponding dimensions of the prostate of the experimental animal are 7·3 by 4·5 mm. The uterus masculinus has the same form as in the experimental common macaque described above, and in its expanded part its maximum diameters are 2 by 1·4 mm. The epithelium of the ejaculatory ducts is thicker than normal, especially in the region immediately above their opening into the urethra, where they show considerable epithelial proliferation, with shedding of the more superficial layers of cells. There is also considerable epithelial growth in the dorsal half of the lower prostatic and succeeding parts of the urethra.

The prostatic glands are not obviously reduced in number. Many are tortuous, a few are dilated, and the glandular epithelium gives some evidence of growth similar to, but not so pronounced as, that in the mona monkey.

As in the case of the mona monkey it is perhaps remarkable that the utricular response of the green monkey to oestrin is purely vaginal in character, for as we have previously described, the uterus masculinus in a very closely allied variety of animal, the Mozambique monkey, is uterine in histological form.

Presbytis entellus: *Hanuman langur*, MM. 56

The uterus masculinus of *Presbytis entellus*, the Hanuman langur of India, differs from that of any other monkey we have investigated experimentally in being a glandular organ in its entire extent. The characters it presents after oestrin treatment are possibly of considerable clinical importance, and a close description is therefore given of the present specimen.

We were unable to secure a control specimen, and for a picture of the normal organ we have to rely on the description given by Heller and Sprinz (1921). In the specimen of langur prostate they figure and describe, the uterus masculinus is a simple glandular diverticulum, somewhat similar to the corresponding human organ. There are regularly branching glandular offshoots from a widely open central lumen, and the organ lies in its own stroma within a fibro-muscular capsule.

The uterus masculinus in our experimental specimen is a highly irregular glandular organ showing many signs of active growth. The total length of the prostate in the fixed preparation, which was serially sectioned, is approximately 8 mm. The length of the uterus masculinus is 2·8 mm. The uterus masculinus ends blindly in a cystic expansion 0·6 mm. in widest diameter. The widest diameter of the prostate is 7·5 mm. Relative to the size of the prostate the uterus masculinus of the langur after oestrin injections is thus far from being an insignificant organ.

At its mouth, which opens slightly to the left of the mid-line on the crista urethralis (the lower two-thirds of which shows some epithelial growth, as does that of the rhesus monkey after oestrin treatment), the uterus masculinus is arranged in a more or less sagittal line of tubular glands, each of which runs cranio-caudally. The individual glands vary much in size, and are typically lined by a single layer of columnar epithelium with basal nuclei and a clear inner cytoplasm. They are set in a matrix of deeper staining epithelial cells generally one to two layers deep. These deeply staining cells show clear signs of active proliferation into the oedematous utricular stroma.

As the sagittal band of uterus masculinus is traced cranially, it develops peninsular outgrowths from the deeper staining basal cells, and within these outgrowths appear the typical columnar celled tubular glands. Slightly cranial to its mid-point, two of these peninsular outgrowths fuse, to enclose within the ring of glandular tissue thus formed a relatively large amount of utricular stroma. From this plane upwards the uterus masculinus is very irregular. The ring of glands disappears by the crowding together of its glandular walls, and by the development of further peninsular outgrowths, which in turn enclose other stromal cells. About 0·8 mm. from its blind end, a single gland becomes cystic, and by its expansion crowds out other glands and forms the blind dilated extremity of the organ.

The glandular epithelium of the uterus masculinus plainly develops from the deeply staining basal cells. Where two glands develop close together they may be separated from each other either by a single layer, or by two or three layers, of these same deeply staining cells, or only by their respective basement membranes. Fusion of two glands may occur by the disappearance of the flattened cells separating them. Many of the glands, apart from the prominent terminal one, are somewhat cystic, and their epithelium is much flattened. The glandular cells are occasionally vacuolated, and the glands usually contain a mass of eosinophilic secretion.

The epithelium of the terminal parts of the common ejaculatory ducts shows many resemblances to the utricular epithelium, but the rest of the prostate has the same essential form, and shows the same changes as a result of the oestrone treatment as does the rhesus macaque. There is no evidence of growth in the true prostatic glands.

DISCUSSION

With the inclusion of the present series, male Primates fall into three groups. Group A, comprising the South American forms, the marmoset and capuchin, shows no clear prostatic reaction to oestrone. In Group B, which includes all but one of the Old World Primates thus far tested, the main prostatic changes are squamous hyperplasia of the uterus masculinus and lower prostatic urethra, together with fibro-muscular growth of the body of the prostate. Group C at the moment comprises only the langur, in which the prostatic reaction is the same as in group B except that the proliferation of the utricular epithelium is glandular and not squamous in type.

In virtue of its morphological form, and by general mammalian analogy, it is impossible to doubt that the uterus masculinus of New World monkeys is homologous with the uterus masculinus of other Primates, and accordingly it seems reasonable to suppose that the lack of any prostatic change in these two forms, following the administration of sufficient oestrin to have such an effect in other Primates, is due to a higher oestrin threshold in the New World primate types. Such a higher threshold exists in the female capuchin (Zuckerman, 1935), but the possibility of a higher threshold existing generally among New World monkeys is not supported by the fact that cystic endometrial hyperplasia resulted from a 14-day course of 100γ of oestrin injected daily into a female marmoset (Russell and Zuckerman, 1936).

Like that of the rhesus monkey, the uterus masculinus of the common macaque, the green monkey and the mona monkey, is presumably homologous only with the vagina of the female, and the utricular response of these forms to oestrin is of little significance to the hypothesis of glandular enlargement of the prostate which we have indicated in the introduction to this paper and elsewhere (Zuckerman and Parkes, 1935). Attention must, however, be paid to the hyperplasia induced in both the green and the mona monkeys in the lower parts of the common ejaculatory ducts, and in the mona monkey in the prostatic tubules themselves. If these apparently oestrin-induced changes are to be brought into line with the similarly provoked change in the uterus masculinus, it is necessary to reconsider briefly the fate of the terminal parts of the Müllerian and Wolffian ducts.

The consensus of present opinion is that at least the distal one-quarter of the solid plate of cells which differentiates into the vagina in the female and the uterus masculinus in the male Primate, develops as an outgrowth of the epithelium of the urogenital sinus, and it is assumed that in becoming part of this solid plate of cells the sinus epithelium replaces cells of Müllerian origin.

The exact amount of epithelial replacement is a matter of dispute. Some authors, for example Vilas (1932), assume that the entire vaginal plate is of sinus origin. In the female the definitive organ derived from this plate, the vagina, is sensitive in its entire extent to oestrin, and given that the embryological facts have been correctly interpreted, it follows that the sinus epithelium as well as the epithelium of the Müllerian ducts is sensitive to oestrin (see Zuckerman, 1936). Since it is not unlikely that the mouths of the Wolffian ducts are also invaded by sinus epithelium at an early stage of development, the epithelial hyperplasia of the terminal parts of the common ejaculatory ducts of our two Cercopitheques thus falls into line with that occurring in the uterus masculinus. The fact that the true prostatic tubules of the mona monkey and the green monkey presented the appearance of an early reaction also assumes a related interest in view of their derivation from the epithelium of the urogenital sinus.

The available data do not allow one to postulate the occurrence of a process of epithelial metaplasia in the true prostatic glands of monkeys similar to that which Burrows (1935) *found in small rodents during the course of prolonged treatment with oestrogenic substances. Thus, the prostatic changes in one of our rhesus monkeys,* MM. 42, *which was injected with* 200γ *oestrone daily for* 89 *days, were simply an exaggerated form of the same type of change occurring after only* 7 *or* 14 *days of similar treatment. For that reason the disorganised glandular hyperplasia of the uterus masculinus of the langur in response to oestrone is especially significant, since it affords an example of a type of initial change which, if continued, might lead to considerable disorganisation of the prostate.*

We are not desirous of over-emphasising the possible significance of this single case, but the facts which have emerged from the comparative study of both rodents and Primates suggest the strong need for the further investigation of the prostatic responses to oestrogenic substances in species of monkey with the type of uterus masculinus of the langur.

SUMMARY

1. The prostate of the marmoset and that of the capuchin show no significant changes in response to injections of oestrone.

2. The most pronounced prostatic changes induced by oestrone in the common macaque, the green monkey, and the mona monkey (like those in the rhesus monkey and the guinea baboon, previously described), are stratification and cornification of the uterus masculinus, and increased general fibromuscular growth.

3. Both the injected green monkey and the injected mona monkey show epithelial growth in the distal parts of the ejaculatory ducts, and the true prostatic glands of the mona monkey show signs of active proliferation.

4. The most significant change in the prostate of the Entellus langur is disorganised glandular hyperplasia of the uterus masculinus. Other prostatic

changes shown by this animal are the same as those in the mona monkey and the green monkey.[1]

5. The clinical significance of the changes in the prostate of the langur is considered in relation to the fact that epithelial metaplasia has not been observed in the true prostatic glands of monkeys during prolonged oestrone injections.

Our best thanks are due to Dr H. M. Carleton for his kind advice on the histological preparations of the langur.

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EXPLANATION OF PLATES

Plate I. *Entellus langur*

Fig. 1. Uterus masculinus of *Entellus langur* after oestrone injections, showing enclosure of stroma between two bands of glandular tubules.

Fig. 2. High-power view, showing the columnar epithelium of the tubular glands, and intraglandular coagulated secretion. Clumps of uncanalised columnar cells can be seen in the deeper-staining basal cells of the utriculus.

Fig. 3. High-power view, showing the breaking down of the intervening wall between two cystic glands.

Plate II

Fig. 1. Prostatic gland of mona monkey after oestrone injections, showing irregular epithelial proliferation, and intraluminar epithelial processes. Two mitotic figures appear in the section.

Fig. 2. Distal part of common ejaculatory duct of green monkey after oestrone injections, showing epithelial proliferation. The structure on the left is the uterus masculinus.

[1] The prostatic and utricular reactions of the drill, *Mandrillus leucophaeus*, to oestrone were tested during the period this paper was passing through the press, and were found to be essentially the same as those of the rhesus monkey.

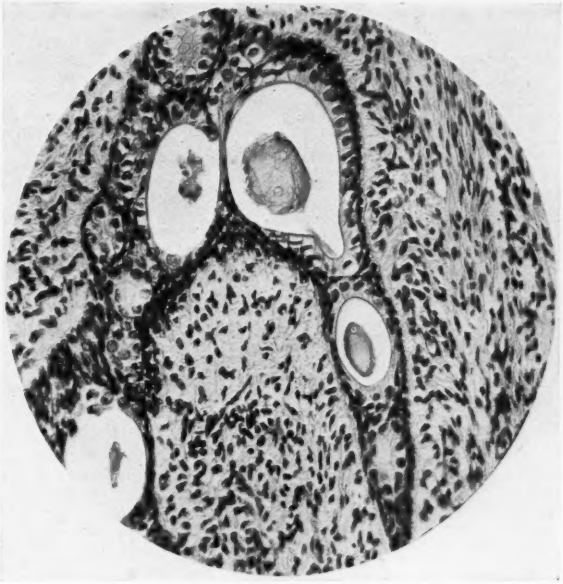

Fig. 2

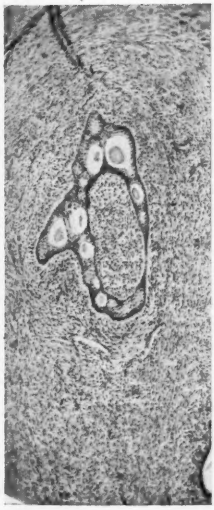

Fig. 1

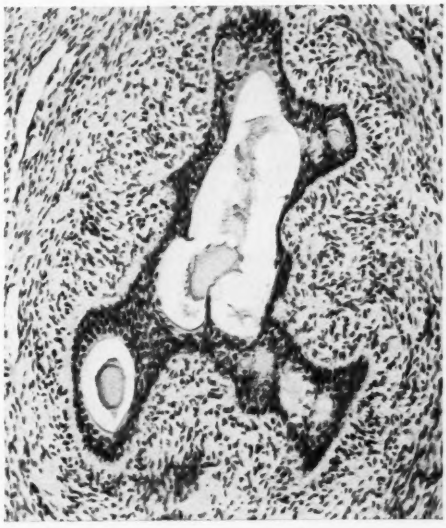

Fig. 3

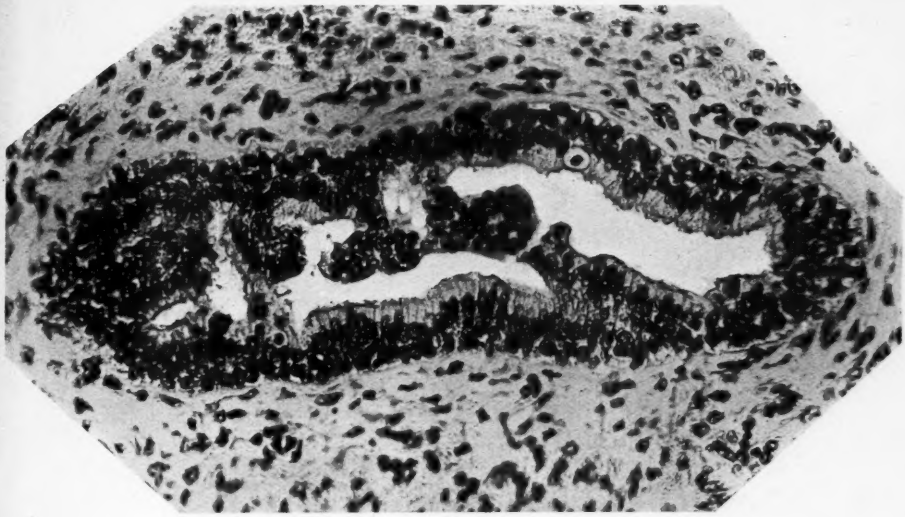

Fig. 1

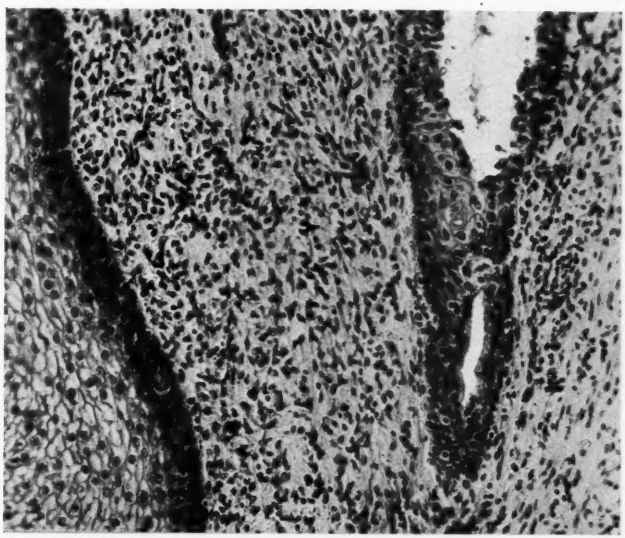

Fig. 2

UNIV. OF MICH.

GROWTH OF THE REPRODUCTIVE AND ENDOCRINE ORGANS OF THE GUINEA-PIG

By RUTH DEANESLY and I. W. ROWLANDS

From the National Institute for Medical Research, Hampstead

INTRODUCTION

During the last two years, a large number of normal guinea-pigs have been killed in this laboratory to obtain control material for various experiments on the reproductive and endocrine organs. The material now covers a wide range of body weight, and in the absence of any extensive information of this kind for the guinea-pig, such as H. H. Donaldson (1924) published for the rat, the data seem worth recording. Ibsen (1928) gave details of pre-natal growth in the guinea-pig, but did not deal with post-natal growth, other than that of the pregnant female. Moore (1932) described the effects of gonadectomy on the weight of the accessory organs of a small group of male and female guinea-pigs, compared with an equal number of normal controls. Kosaka (1932) analysed the relations of cortex and medulla in the adrenals of sixteen adult guinea-pigs and reports that the left gland is always larger than the right.

No attempt is made here to deal with the histology or functional activity of the reproductive and endocrine organs of the guinea-pig, since numerous papers on these subjects have already been published.

MATERIAL AND TECHNIQUE

Animals. One hundred male and ninety-one female guinea-pigs were examined. The males ranged in body weight from a little over 200 gm. to a little over 700 gm.; the females from 170 to 700 gm. The body weights recorded (B.W.) are those of the intact animals, of which the great majority were from the inbred colony maintained at the Institute's Farm Laboratories. Some animals from other sources were included for completeness.

Preservation and weighing. The reproductive organs and the thyroids and adrenals were removed from each animal and fixed in Bouin's fluid and upgraded to 70 per cent. alcohol. The testes, seminal vesicles, ovaries, thyroids and left and right adrenals were then weighed on a torsion or chemical balance. The epididymis and prostate were not weighed, being difficult to dissect accurately in the guinea-pig. Weighing after fixation was unavoidable in the case of the seminal vesicles, which are distended with fluid in the fresh condition, and it was convenient for the other organs. Occasionally, however, some were damaged or put into different fixatives and not weighed, so that

the available data for the separate organs are slightly fewer than the total numbers of animals.

Treatment of results. The results are shown in the form of scatter diagrams. No mathematical treatment has been attempted, except for the thyroids (Rowlands and Parkes, 1934), although in several cases the organ weights bear an obvious linear relation, or two successive linear relations, to the body weight, and the calculation of regression lines would have been a simple matter. The additional accuracy obtainable, however, by this as compared with graphical methods, would be of no value in the circumstances.

TESTES

Fig. 1 gives the scatter diagram for the weights of the paired testes which show three fairly distinct phases of growth. At B.W. under 300 gm. the testes are small, mainly below 0·5 gm., and the rate of increase is slight. From

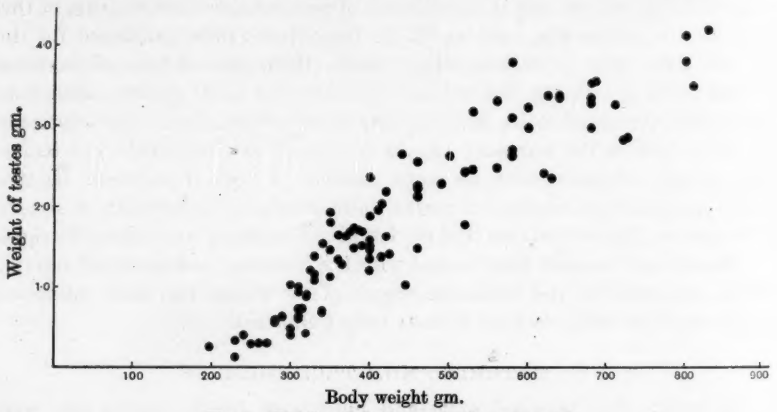

Fig. 1. Weight of the testes in the guinea-pig.

300 to 400 gm. B.W. there is rapid growth, until the testes reach an average of about a little over 2 gm. From this point, growth is slower, but, nevertheless, the testes may approach a weight of 4 gm. in large guinea-pigs of over 800 gm. The variation in testis size is not unduly great considered in relation to variations in B.W.

SEMINAL VESICLES

The scatter diagram for seminal vesicle weights is given in fig. 2. The seminal vesicles, instead of remaining undeveloped during the early growth of the testes, as in some other species, begin to enlarge at about the same time as the testes. At B.W. below 300 gm. the seminal vesicles are small, generally below 0·25 gm., but thereafter their development is rapid. From 300 to 350 gm. B.W. they may weigh anything from 0·1 to 1·5 gm., showing a wide

range of variability even for a rapidly growing organ. This gross variability increases comparatively little as the B.W. goes up to 600 gm., so that the percentage variability actually decreases. In about 600-gm. guinea-pigs and larger ones, however, the weights observed were extremely variable, roughly from 1 to 10 gm. Only two conclusions can be drawn from these data: (*a*) that the vesicles are normally small and undeveloped below 300 gm. B.W. and (*b*) that only in animals of over 500 gm. B.W. is there a reasonable certainty that the vesicles are well grown.

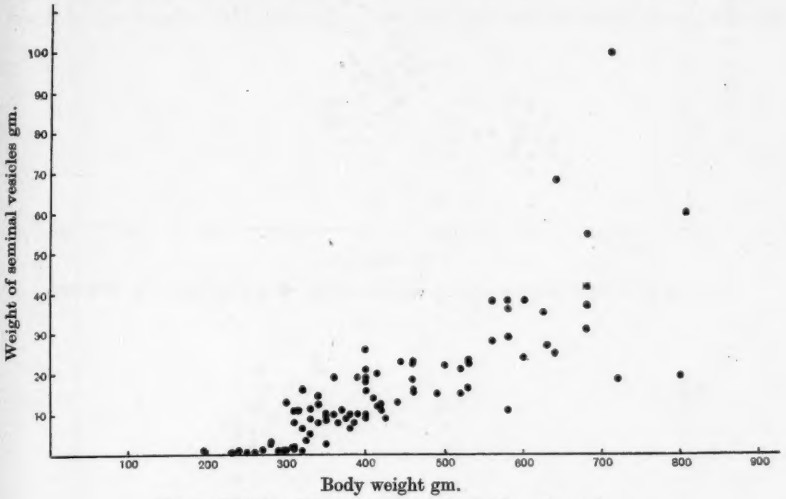

Fig. 2. Weight of the seminal vesicles in the guinea-pig.

OVARIES

The scatter diagram for ovary weights is shown in fig. 3. The general trend is very similar to that of the testes. From 150 to 300 gm. B.W. the ovary weight is remarkably steady, being about 50 mg., with a gross variation of ±about 15 mg. Between 300 and 400 gm. B.W. the ovaries may increase appreciably in weight, but many as low as 50 mg. are still found. Above 450 gm. B.W. the numbers are inadequate for drawing conclusions.

The onset of ovarian activity, as diagnosed by weight, at 300 gm. B.W. obviously corresponds to the start of the oestrous cycle. The smallest pregnant animal weighed 340 gm. The rise in ovary weight at puberty, however, is not nearly as steep as in the rat (H. H. Donaldson, 1924), where the corpora lutea form a larger proportion of the ovary. It is very noticeable in these records that the ovaries of pregnant animals are no larger than those of non-pregnant, even allowing for the slight misplacement of the animals in the weight scale; this would be expected from the small size of the corpora lutea of pregnancy (Deanesly and Parkes, 1931).

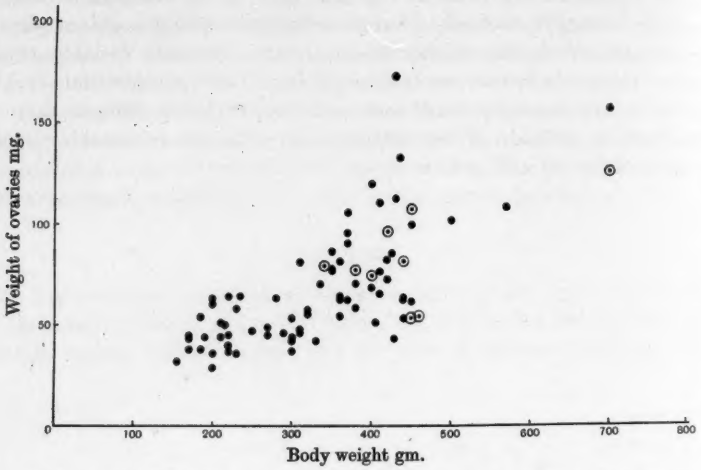

Fig. 3. Weight of the ovaries in the guinea-pig. ● non-pregnant; ⊙ pregnant.

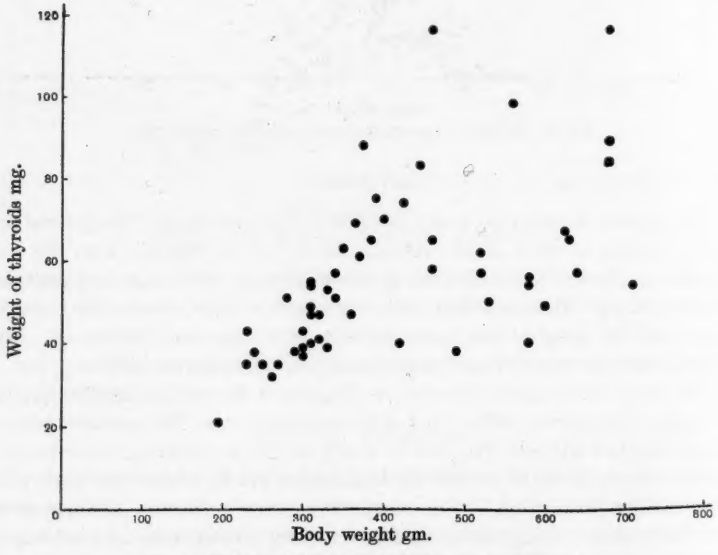

Fig. 4. Weight of the thyroids in the male guinea-pig.

THYROIDS

The thyroids of the males are plotted in fig. 4 and those of the females in fig. 5. Growth in the female up to 300 gm. B.W. has been dealt with by Rowlands and Parkes (1934), who found that the obviously linear relationship could be expressed by the formula $y = 0{\cdot}159x - 1{\cdot}02$, where x = B.W. in gm. and y = weight of the thyroids in mg., i.e. that the thyroids increased about 1 mg. in weight for every 6 gm. B.W. In the male, a good linear relationship is found up to 350 gm. B.W., with a very similar slope and position to that found in the female. In the female above 300 gm. and the male above 350 gm.

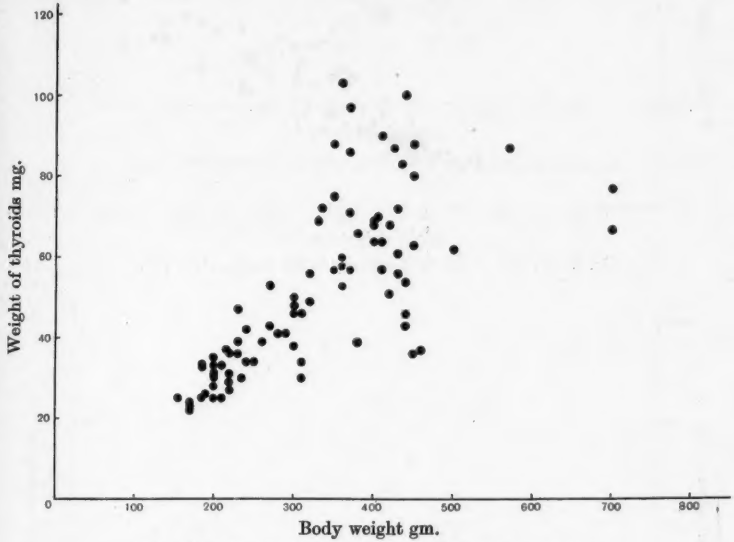

Fig. 5. Weight of the thyroids in the female guinea-pig.

B.W. the points fan out so greatly that it is difficult to draw conclusions except that the thyroid of the adult guinea-pig is very variable. A male guinea-pig of 700 gm., for instance, may have thyroids only half the size of another guinea-pig of only 450 gm.

ADRENALS

Male guinea-pigs. Separate scatter diagrams are given for the left and right adrenals. Those for the male (figs. 6 and 7) provide two definite conclusions:

(*a*) Adrenal weight bears a linear relation to B.W. Over the wide range of B.W. examined each adrenal increases by about 4 mg. for each 10 gm. increase in B.W.

(*b*) The left adrenal is slightly but consistently heavier (10–20 mg.) than the right adrenal (cf. Kosaka, 1932). This is also true of the rat (J. C. Donaldson, 1919).

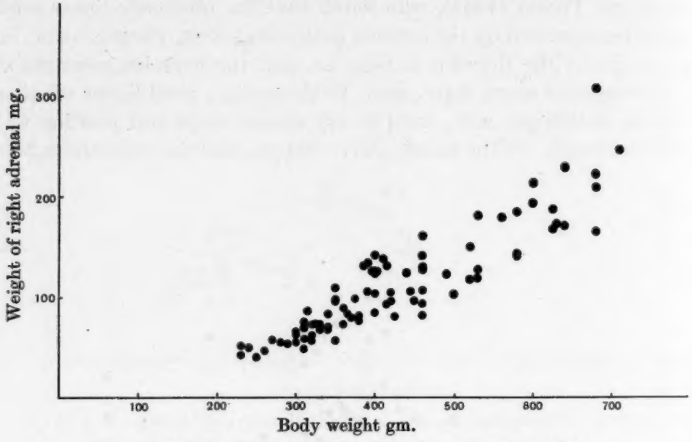

Fig. 6. Weight of the right adrenal in the male guinea-pig.

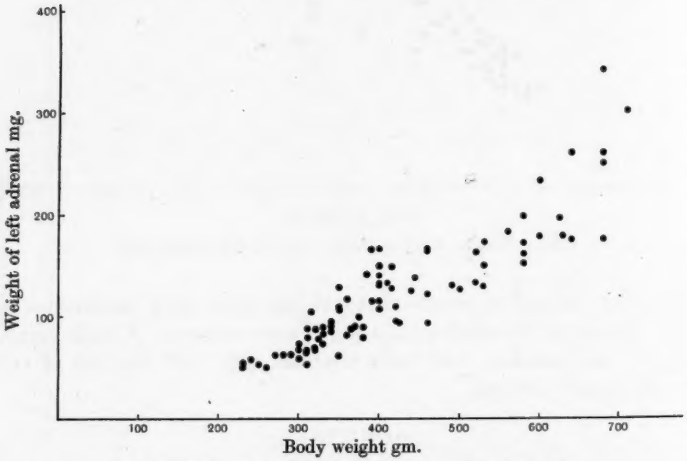

Fig. 7. Weight of the left adrenal in the male guinea-pig.

Female guinea-pigs. The scatter diagrams for the adrenals (figs. 8, 9) show that their growth is rather different in the female, although, as in the male, the left adrenal is the larger. There is a definite acceleration in the growth of both adrenals when the B.W. reaches 320–340 gm.; i.e. at exactly the same

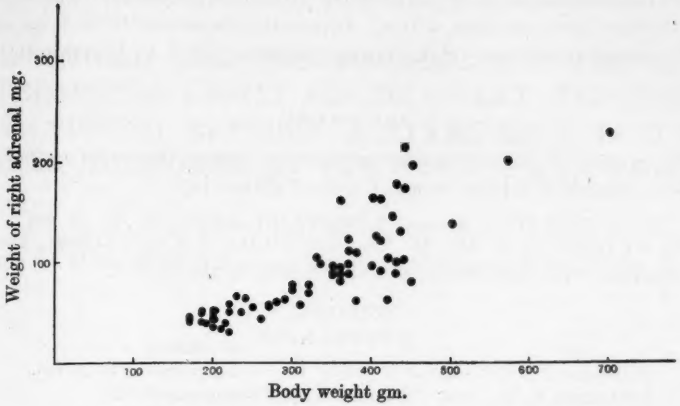

Fig. 8. Weight of the right adrenal in the female guinea-pig.

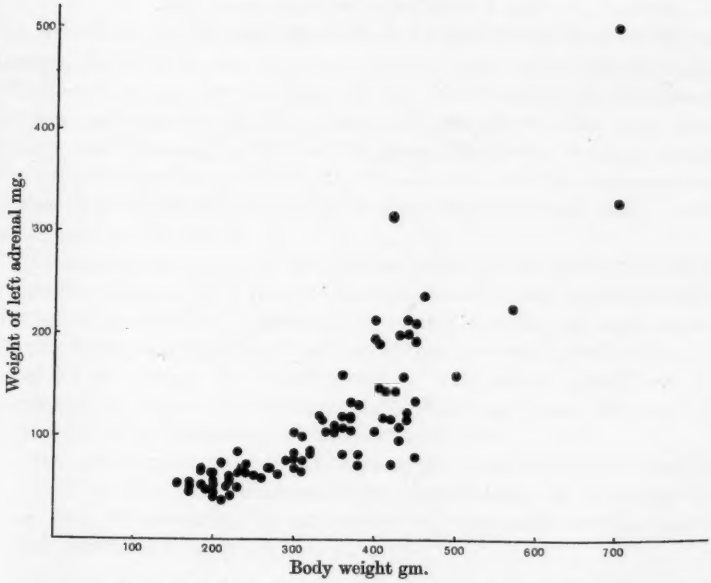

Fig. 9. Weight of the left adrenal in the female guinea-pig.

B.W. as that associated with the sudden increase in the ovaries. Adrenal growth therefore seems to accompany the attainment of sexual maturity when the pituitary becomes more active. Apparently, however, there is no appreciable increase in adrenal weight during pregnancy (cf. J. C. Donaldson, 1924).

SUMMARY

The growth of the testes, seminal vesicles, ovaries, thyroids and adrenals has been studied in a large series of normal guinea-pigs.

We are indebted to Miss M. Allanson, Ph.D., of King's College, London, for assistance with the dissection and weighing of the organs.

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ANATOMICAL AND HISTOLOGICAL STUDIES OF AN INTERSEXUAL LESSER SHREW (*SOREX MINUTUS* L.), WITH SPECIAL REFERENCE TO THE EFFECTS OF THE MALE HORMONES ON THE UTERUS AND VAGINA

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I. INTRODUCTION

The occurrence of an intersexual lesser shrew seems to be of sufficient interest to record. So far as we are aware there is no record of intersexuality in shrews in the literature, nor have we observed any other example among upwards of one thousand common and three hundred lesser shrews that have been dissected in this laboratory in the last few years. Moreover, there do not appear to be any records of intersexuality in other insectivores if the various attempts, the most recent by Matthews (1935), to show that the female mole is normally intersexual, are discounted.

This intersex is of interest also in other more general respects. Both male and female organs are as a rule non-functional and incompletely developed or abnormal in mammalian intersexes, but in this example the male organs are fully developed and functional and the uterus is as well developed as in the normal female during the breeding season. It therefore provides a unique opportunity for examining the effects on the Fallopian tubes, uteri and vagina of the endocrine environment of the functional male.

It also admits of investigating, from an angle other than the embryological, the extent to which the Müllerian ducts contribute to the formation of the vagina, and of comparing the anatomical relations and vascular supply of the male and female organs in the one subject.

II. DESCRIPTION

The specimen (S 1818) was trapped on May 10, 1935, at Tan-y-marian, near Bangor. It weighed 3·8 gm. total body weight. The reproductive organs were dissected out in one piece and were fixed in the alcoholic modification of

Bouin's fluid. The intersexual character of the organs was not observed until afterwards, so that no information is available regarding the condition of the mammary glands. Since the animal obviously was mature it must have been born in the previous season (Brambell, 1935[1]), and was therefore between 7 and 13 months old.

The reproductive organs are shown in their natural relations in Plate I, fig. 1 (ventral) and Plate I, fig. 2 (dorsal). They are spread out so as to display the various organs from the ventral and dorsal aspects respectively in Plate II, figs. 1 and 2. The parts can be identified by means of the outline keys to these four figures. The male organs were all present and exhibited the full development characteristic of the adult male at the height of the breeding season. The testes had descended normally on each side into the scrotal sacs, which in the male shrew open freely into the peritoneal cavity. The penis was well developed and exhibited the characteristic sigmoid flexure of the retracted organ. The prepuce was normal. It is therefore probable that the external genitalia were similar to those of a normal adult male. The testes, epididymides and vasa deferentia were normal in size and structure, the latter exhibiting the small upper and very large lower swellings that are found in adult shrews (Ärnbäck-Christie-Linde, 1907; Brambell, 1935). The prostate glands were moderately well developed and normally disposed. The glands of Cowper were large, even for an adult male, and were otherwise normal. That on the right had been slightly damaged so that its outline appears irregular in the illustrations. Cowper's glands are represented in the female by the glands of Bartholin, which in the lesser shrew cannot be distinguished macroscopically. These organs in this intersex were therefore definitely male in character.

The female organs present were Fallopian tubes, uteri and vagina; no ovaries were found. The Fallopian tubes extended along the whole length of the epididymides to the actual ventro-lateral borders of which they were closely attached. They can be seen in Plate I, fig. 2, and Plate II, fig. 1. They were thus abnormally extended, presumably owing to the great size of the testes, as compared with the condition in the normal female where they are lapped around the small ovaries. The two uterine cornua were united in the middle line where the common cervical canal opened into them as in the normal female. The right cornu was slightly longer than the left, whereas they are normally of equal length in the female. The lateral extremity of each cornu, where the Fallopian tube opened into it, was closely applied to the surface of the globus minor of the epididymis. The uterus was situated antero-ventrally to the upper swellings of the vasa deferentia, which were in the broad ligaments. The uterus as a whole was larger than that of a normal non-parous adult female in late oestrus and presented a similar appearance macroscopically, as though it were hyperaemic and partly distended with fluid. The vagina was situated dorsally and median to the lower swellings of the vasa

[1] Unpublished work on the lesser shrew shows that it resembles the common shrew in not reaching maturity in the season in which it is born.

deferentia, to the dorso-median surfaces of which it was closely applied throughout the posterior four-fifths of its length. Anteriorly, where it joined the uterus, it was not applied to the vasa deferentia, which diverged to right and left. The anterior extremity of the vagina was almost as large as in the normal late oestrous female, but posteriorly it tapered continuously and appeared macroscopically to end at the level of the neck of the bladder where the two vasa deferentia met in the middle line.

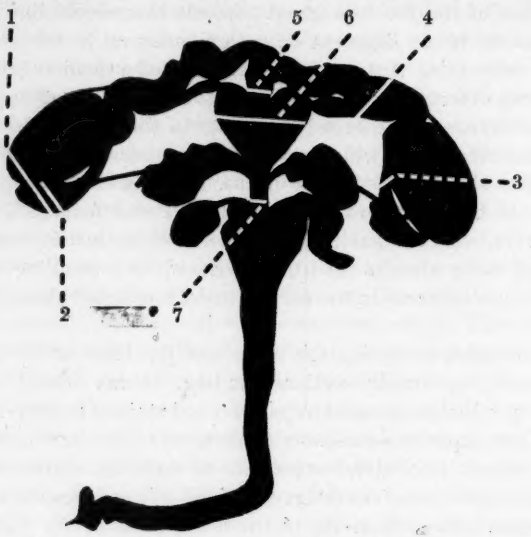
The spermatic artery and vein left the testis at the point where the body of the epididymis joined the globus major and ran from there to cross the ventral surface of the prostate gland towards the middle line. The uterine vessels ran in the broad ligament on each side lateral to the lower swellings of the vasa deferentia. Anteriorly each forked, the median branch running around the vas deferens to the region of the cervix. The other branches ran diagonally outwards in the broad ligaments to the extremities of the uteri, giving off numerous vessels which ran to the vasa deferentia and forked around them on either side to supply the uterus. The vascular supply was thus a combination of that found in the normal male and female. The spermatic artery and vein, with the pampiniform plexus of the latter, resembled those of the normal male, whereas the uterine vessels, corresponding to the vessels supplying the vas deferens in the normal male, resembled those of the normal female.

All the material, excepting the penis and the lobes of the prostate and Cowper's glands, was serially sectioned at 10 μ. It was cleared in cedar-wood oil followed by toluol, embedded in paraffin and stained in Mayer's haemalum and eosin. Three separate series were made so as to obtain transverse sections of the chief organs; two lateral series, one on each side approximately transverse to the uterine cornu, vas deferens, epididymis and long axis of the testis, and one median series transverse to the lower parts of the vasa deferentia, vagina and urethra. The approximate positions and planes of the sections figured can be seen by reference to text-fig. 1.

Both gonads were found to be normal testes exhibiting active spermatogenesis (Plate III, fig. 1). No trace of ovarian tissue was found associated with the testes or elsewhere. The epididymides also appeared normal (Plate III, figs. 1 and 3), and many of the tubules were crowded with spermatozoa. The upper swellings of the vasa deferentia, situated in the mesometrium close to the uterus (Plate III, fig. 4), and the larger lower swellings in the broad ligament on each side of the vagina (Plate IV, fig. 3) were well developed and their lumina were crowded with spermatozoa. Spermatozoa were present also in some of the alveoli which, opening into and surrounding the lumen, form the greater part of the lower swellings (Plate IV, fig. 3). The histological appearance of the prostate glands also was similar to that in a normal functional male, the alveoli being large and filled with secretion.

An ostium tubae was present at the cephalic pole of each testis, firmly attached on the one side to the tunica albuginea of the testis and on the other

to the globus major of the epididymis (Plate III, fig. 1). These ostia opened freely into the peritoneal coelom. Thus their anatomical relations are similar to those in an hermaphrodite pig described and figured by Krediet (1931). Histologically they resembled those of a normal female lesser shrew. The Fallopian tube on each side extended from the ostium around the tip of the globus major and along the actual ventro-lateral border of the epididymis to the tip of the uterine cornu, which was closely applied to the globus minor. The Fallopian tube was enclosed within the connective tissue sheath of the epididymis (Plate III, fig. 1) on the side next the testis. The distal part of the



Text-fig. 1. Silhouette of the reproductive organs from the dorsal side showing the positions of the sections figured in the Plates. The continuous white lines mark the positions and directions of the sections and the numbers attached by the dotted guide lines refer to the figures of these. 1 = Plate III, fig. 1; 2 = Plate III, fig. 2; 3 = Plate III, fig. 3; 4 = Plate III, fig. 4; 5 = Plate IV, fig. 2; 6 = Plate IV, fig. 3; 7 = Plate IV, fig. 5.

tube was wider (Plate III, fig. 2) than the proximal (Plate III, fig. 3) and exhibited the epithelial crypts and folds which complicate the lumen in this region in the normal female. The lower part of the tube, also as in the normal female, had a relatively simple lumen with only shallow epithelial folds and a much thicker muscular sheath. The Fallopian tubes resembled in structure, apart from the fact that they were stretched out owing to the large size of the testes and the length of the epididymides, those of normal females. Their epithelium did not appear as active nor was their diameter as great as in an adult female during the breeding season.

The uterine lumen was found to be distended with fluid and nearly circular in cross-section, thus increasing the external diameter of the uterus. Cross-

sections of the uterine cornua (Plate III, fig. 4) measured approximately 2·0 × 1·5 mm. as compared with 1·5 × 1·0 mm. in the normal adult female in oestrus, but the area of tissue in the two was approximately equal. The uterus, if it had not been distended, would have been quite as large as in a normal oestrous female.

The coagulated fluid in the lumen was full of small granules which stained with eosin and contained a few scattered leucocytes and debris of epithelial cells. Its appearance suggested that it was composed mainly of the secretion of the uterine glands. The uterine epithelium (Plate IV, fig. 1) was composed of a single layer of epithelial cells, almost cuboidal on the mesometrial side and columnar antimesometrially. Their nuclei were oval or elongated and arranged in a single layer as in the normal oestrous female. The uterine glands were numerous antimesometrially and extended some distance round the sides, as occurs normally during early pregnancy. They were more numerous in cross-sections than is normally the case in the non-pregnant uterus. The uterine glands as a rule in non-pregnant animals have little or no lumen. They only develop lumina after oestrus when colloid appears in them. Yet all the glands in this intersex exhibited distinct lumina, in many cases much enlarged and containing colloid (Plate IV, fig. 1). The distension of the lumina of the glands resulted in their epithelia being cubical or even flattened. Thus in their number, the possession of distinct lumina and the presence of colloid they resemble those of the normal female in late oestrus or early pregnancy. They differed in that their lumina were more distended than in the normal female, so that the antimesometrial mucosa had a "Swiss-cheese" appearance similar to that described and figured by Parkes (1935) as experimental endometrial hyperplasia, produced by prolonged injections of oestrin, in the mouse. The muscular layers of the uterine wall were as well developed as in the normal animal. Medially the lumina of the two cornua were confluent where the common cervical canal opened into them.

Anteriorly the vagina is approximately 1·3 mm. in diameter (Plate IV, fig. 2) and nearly circular in cross-section. Laterally it is not attached directly to the vasa deferentia but to the broad ligament in this region, but the vasa deferentia soon curve inwards on each side and become attached to it (Plate IV, fig. 3), the vagina lying dorsally in the angle between them. It tapers gradually to the posterior extremity where it measures only 67 μ in diameter (Plate IV, fig. 5). About the level of the neck of the bladder the vagina and the vasa deferentia are surrounded by a common muscular sheath, and below the bladder the urethra and the ducts of the prostate glands also become included in the sheath (Plate IV, fig. 5). Anteriorly the lumen of the vagina is distended with the granular secretion of the uterine glands, but the longitudinal folding of the mucosa, characteristic of this region of the shrew vagina, can be distinguished in transverse sections as ridges projecting into the lumen. Throughout its length the vaginal epithelium is thin, consisting of one or two layers of flattened or cubical epithelial cells (Plate IV, fig. 4) which

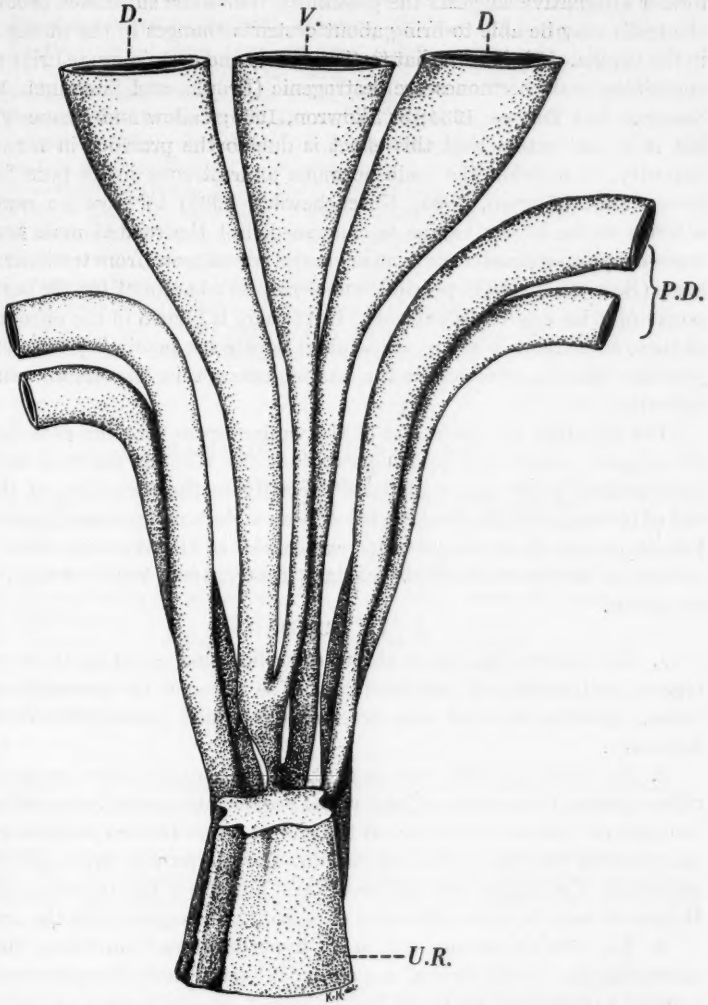
show no signs of cornification. The vaginal epithelium appears quite inactive, more so than that of an adult female even in anoestrus, and resembles that of a very young shrew before the beginning of puberty. The resemblance of the epithelium to that of the prostatic utricle or uterus masculinus of the male rhesus monkey described and figured by Parkes and Zuckerman (1935) is striking. Comparison of Plate IV, fig. 5, with their fig. 2 (A) (*ibid.*) shows this similarity. The inactive condition of the vaginal mucosa is thus in striking contrast to the hyperplasia of the uterine mucosa.

The details of the relations of the various ducts and of their openings into the urethra were investigated by means of a wax-plate reconstruction of the region from the base of the bladder to the crus penis. It can be seen from this model (text-fig. 2) that the vagina, which has a lumen throughout its length, opens into the left vas deferens almost immediately before this joins the right, and both open by a common aperture into the ventral wall of the urethra. The prostatic lobes open into the urethra, by means of two ducts on each side, a few sections posterior to the opening of the vasa deferentia.

III. CONCLUSIONS

The presence of the Fallopian tubes, uterus and vagina together with well-developed and obviously functional male organs renders this specimen a very beautiful example of intersexuality and, we believe, the only one recorded hitherto in a shrew. However, by far the most interesting problem presented by it lies in the condition of the uterus and vagina and the physiological causes which have brought it about. The fact that the male organs are functional and have attained the maximum development characteristic of the breeding season, coupled with the absence of ovaries, implies that the endocrine conditions must approximate to those of a normal male. Yet this male hormonal environment has caused the uterus to develop to a stage characteristic of that of the normal adult female, and has maintained it in what may be called a hyper-oestrous condition resembling the hyperplasia brought about by prolonged experimental administration of oestrin (Parkes, 1935). The vagina, on the other hand, although it is nearly as large as that of a normal adult female anteriorly, tapers away posteriorly and is lined throughout by a thin and very inactive epithelium. The vaginal epithelium is thinner and appears less active than that of an adult female at any stage of the oestrous cycle, resembling more closely the epithelium of the uterus masculinus found in the males of some species (Parkes and Zuckerman, 1935).

It is well known that the males of several, possibly all, species of mammals produce and excrete varying quantities of oestrogenic substance. The oestrogenic substance in the stallion actually appears to be oestrone (Zondek, 1934). Oestrogenic substance, therefore, was probably present in this intersex and might produce the uterine hyperplasia. But if it did it might have been expected to produce vaginal cornification also. The absence of vaginal cornification indicates that either (*a*) oestrogenic substance was not present in



Text-fig. 2. Diagram of wax-plate reconstruction of the vasa deferentia, vagina, prostatic ducts and urethra in the region where they join. The urethra is cut short to expose the other ducts. Plate IV, fig. 5, is of a transverse section through the middle of this region. For key to guide letters see p. 347. ×67.

sufficient quantity or (*b*) that its action on the vagina was inhibited. The former alternative suggests the possibility that some substance produced by the testis may be able to bring about oestrous changes in the uterus but not in the vagina. It is known that testis extracts and certain crude urine extracts containing male hormones are oestrogenic (Brouha and Simonnet, 1928; St Skowron and Turyna, 1934; St Skowron, 1934; Callow and Deanesly, 1935); but it is not certain that this effect is due to the presence in them, as an impurity, of oestrin. The male hormone androsterone in its pure form has been shown (Warren, 1935; Korenchevsky, 1935) to have no oestrogenic activity so far as the vagina is concerned, but the related male hormones, trans-dehydro-androsterone from urine and testosterone from testis, are oestrogenic (Butenandt, 1935), possibly sufficiently so to account for the oestrogenic power of urine and testis extracts. Until more is known of the effects, if any, of these substances in the ovariotomised female the possibility that they may produce oestrous changes in the uterus but not in the vagina cannot be excluded.

The fact that the lower end of the vagina opens into the vasa deferentia at the point where they join suggests that the Wolffian ducts as well as the urino-genital sinus may contribute normally to the formation of the lower end of the vagina in the female. It has been maintained frequently on embryological grounds that the posterior extremities of the Wolffian ducts do contribute to the formation of the vagina (Baxter, 1933, 1934), at least in some mammals.

IV. SUMMARY

1. The intersexual lesser shrew described had a complete set of male organs, well developed and functional. The presence of spermatozoa in the testes, epididymides and vasa deferentia provided presumptive evidence of fecundity.

2. No ovarian tissue was present. The Fallopian tubes were normally differentiated into proximal and distal regions; the ostia tubae, attached to the cephalic pole of the testis and the globus major of the epididymis on each side, opened into the peritoneal coelom. The uterus was large and distended with fluid. The vagina was well developed anteriorly but tapered posteriorly. It opened into the vasa deferentia close to their junction with the urethra.

3. The uterine mucosa was in a "hyper-oestrous" condition, the glands presenting the "Swiss-cheese" appearance characteristic of experimental endometrial hyperplasia produced by prolonged administration of oestrin. The vaginal epithelium was thin and very inactive. The possible physiological significance of these phenomena is discussed.

We are indebted to Dr A. S. Parkes, F.R.S., for his advice and criticism. The expenses of this research were defrayed in part by a grant from the Government Grant Committee of the Royal Society to one of us (F. W. R. B.), for which we wish to express our thanks.

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EXPLANATION OF PLATES I–IV

Key to lettering

B.	Bladder.	*O.*	Ostium tubae.
C.	Cowper's gland.	*P.*	Penis.
D.	Vas deferens.	*P.D.*	Prostatic duct.
D.A.	Upper swelling of vas deferens.	*P.R.*	Prostate gland.
D.B.	Lower swelling of vas deferens.	*S.V.*	Spermatic vessels.
E.	Epididymis.	*T.*	Testis.
E.A.	Globus major of epididymis.	*U.*	Uterus.
E.P.	Globus minor of epididymis.	*U.R.*	Urethra.
F.	Fallopian tube.	*U.V.*	Uterine vessels.
M.	Circular muscle.	*V.*	Vagina.

PLATE I

Fig. 1. Photograph of the ventral aspect of the reproductive organs in their natural relations to each other. The greater length of the right uterine cornu as compared with the left is shown. A keyed diagram is provided. ×4.

Fig. 2. Photograph of the dorsal aspect of the reproductive organs in their natural relations to each other. The Fallopian tubes can be seen on the borders of the epididymides next to the testes and the vagina between the lower swelling of the right vas deferens and the left prostatic lobe. A keyed diagram is provided. ×4.

PLATE II

Fig. 1. Photograph of the ventral aspect of the reproductive organs displayed. A keyed diagram is provided. ×2·6.

Fig. 2. Photograph of the dorsal aspect of the reproductive organs displayed. A keyed diagram is provided. ×2·6.

PLATE III

Fig. 1. Photomicrograph of a transverse section through the testis and epididymis near the globus major. The ostium tubae can be seen attached to the epididymis and tunica albuginea and opening freely into the peritoneal coelom. The distal part of the Fallopian tube is included in the sheath of the epididymis. The spermatic tubules are in active spermatogenesis. ×25.

Fig. 2. Photomicrograph of a transverse section of the distal part of the Fallopian tube showing the characteristic epithelial crypts and folds which complicate the lumen in this region. ×204.

Fig. 3. Photomicrograph of a transverse section of the proximal part of the Fallopian tube, showing the thick muscular wall and relatively simple lumen, and of a neighbouring tubule of the epididymis crowded with spermatozoa. ×273.

Fig. 4. Photomicrograph of a transverse section of the uterus and of the upper swelling of the vas deferens in the mesometrium. The uterine lumen can be seen to be distended with fluid and the vas deferens with spermatozoa. The enlarged glands in the mucosa are situated antimesometrially. ×31.

PLATE IV

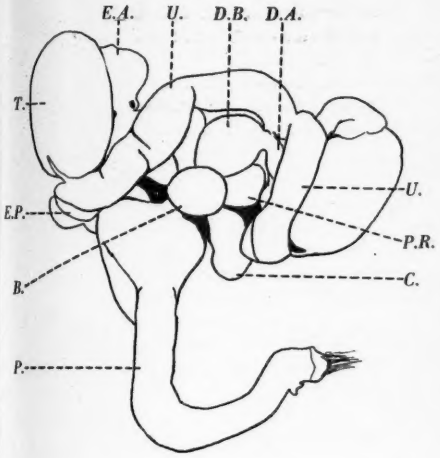
Fig. 1. Photomicrograph of a transverse section of part of the antimesometrial uterine mucosa shown in Plate III, fig. 4. The columnar uterine epithelium and the distended glands, lined by cubical or flattened epithelium, some of them containing colloid, can be seen. ×340.

Fig. 2. Photomicrograph of a transverse section of the anterior end of the vagina showing the longitudinal epithelial folds and the distended lumen. ×45.

Fig. 3. Photomicrograph of a transverse section of the vagina, posterior to that shown in fig. 2, and of the lower swellings of the vasa deferentia. The vagina lies dorsally between the vasa deferentia. The central canal of the vas deferens is filled with a dark mass of spermatozoa and is surrounded by the spongy mass of alveoli. ×16.

Fig. 4. Photomicrograph of part of a transverse section of the vagina showing the thin vaginal epithelium consisting of flattened or cubical nucleated cells arranged about two rows thick. The epithelial cells show no signs of cornification. ×485.

Fig. 5. Photomicrograph of a transverse section of the urethra and of the genital ducts a few sections anterior to where they open into it. The vagina is very small and lies between the vasa deferentia. There are two prostatic ducts on each side. All the ducts are included in a common sheath formed by the urethral musculature. ×75.



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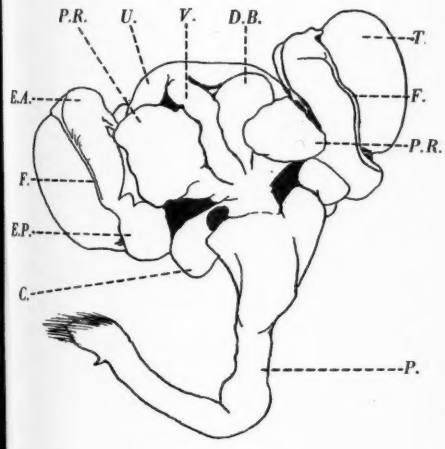

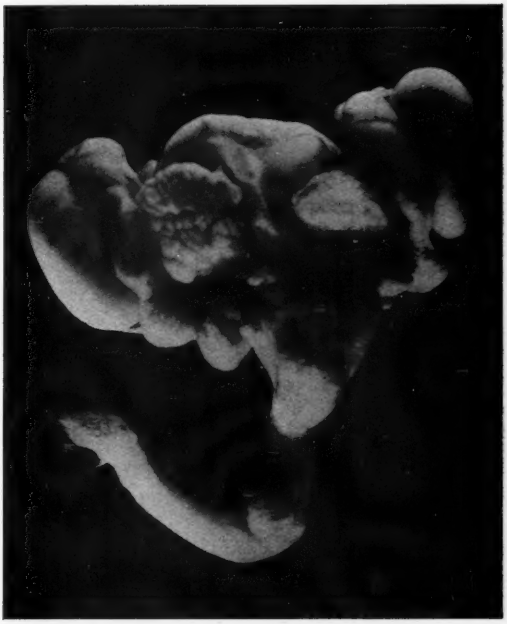

Fig. 2

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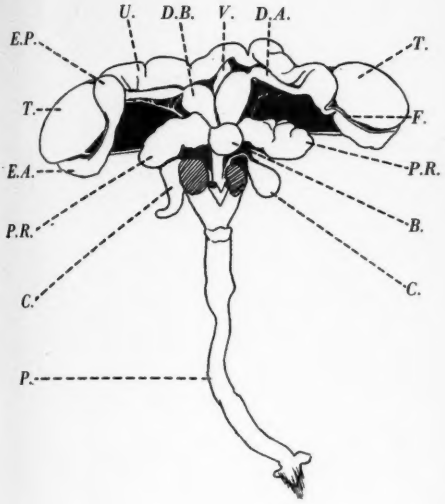

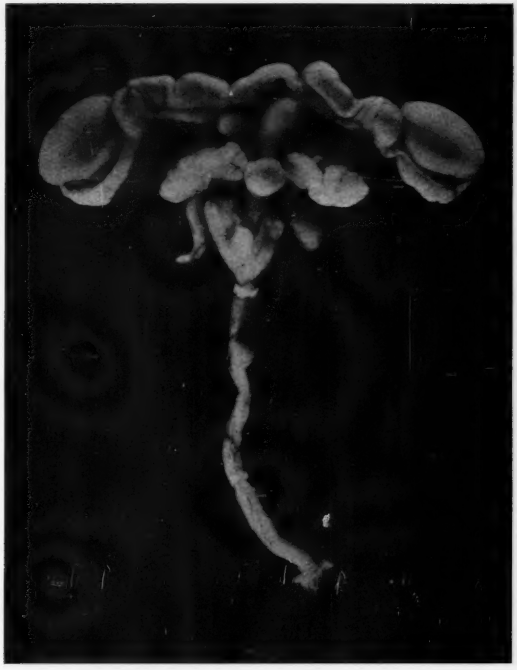

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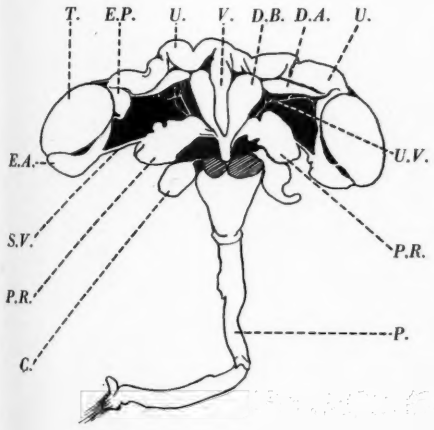

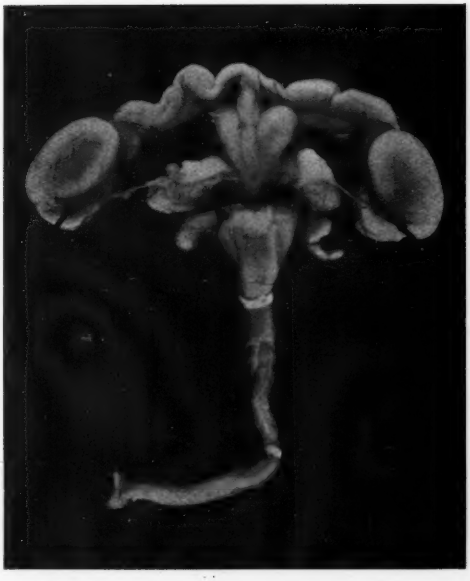

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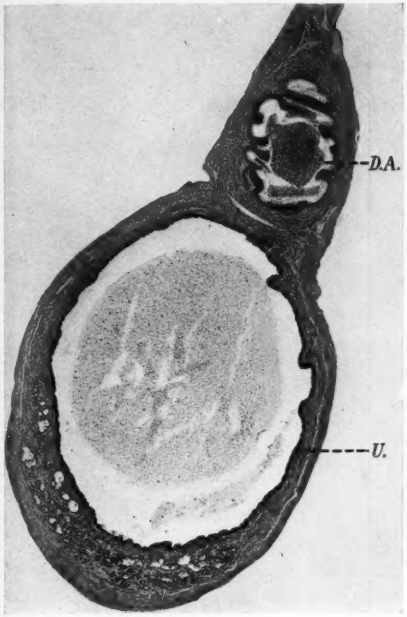

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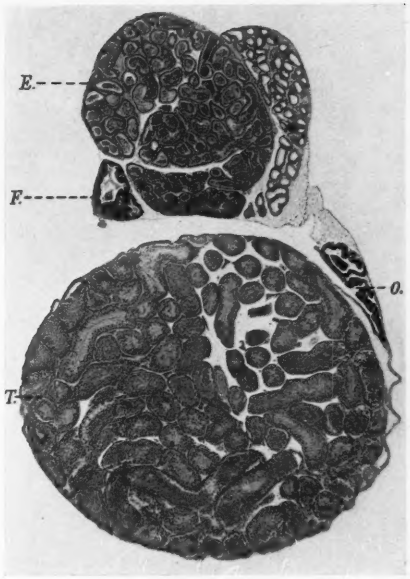

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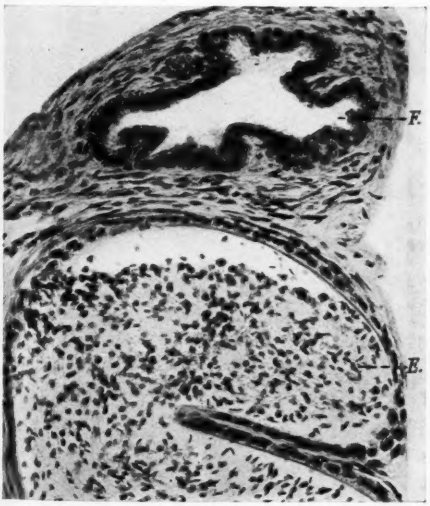

Fig. 3

Fig. 2

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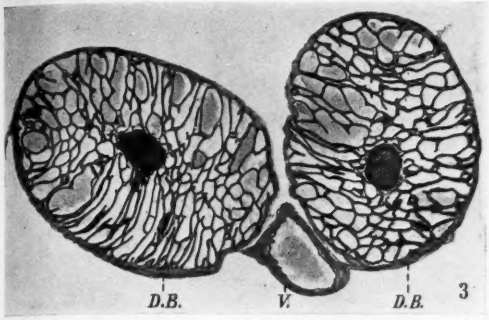

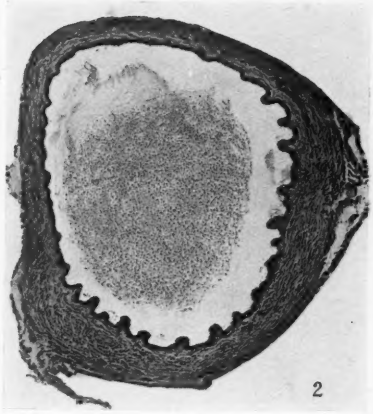

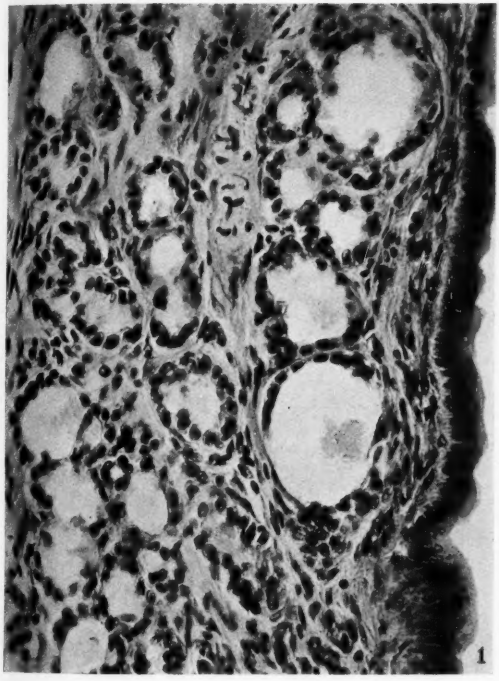

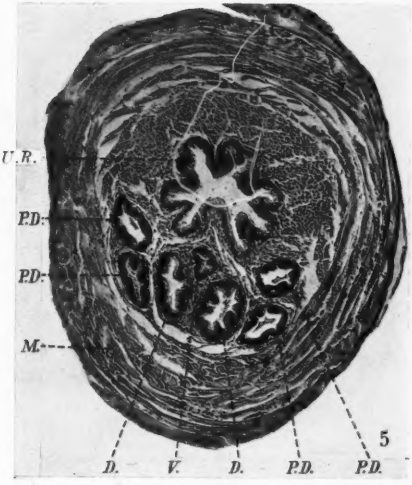

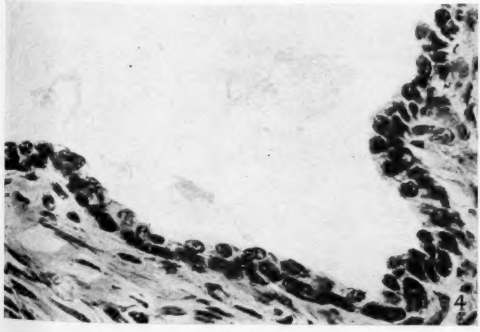

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THE INNERVATION OF THE DENTINE

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The problem of the exact termination of the nerves which run in the pulps of the teeth has been the cause of a considerable amount of discussion for many years. Workers in this field can be divided into two schools, those who believe that the nerve fibres terminate in the odontoblast layer and those who claim to have traced them into the dentine itself. It is not considered necessary in this paper to review the earlier literature as this has already been done on many occasions, and the authors are of the opinion that until recent years no satisfactory evidence has been forthcoming that the nerve fibres could be demonstrated within the dentine.

Recently, however, important contributions have been made by two independent workers, Sealey and Tojoda, who have published very convincing evidence that the nerve fibres from the pulp do actually terminate in the dentine.

Sealey (1) worked with teeth fixed in formol saline, decalcified in hydrochloric and nitric acid and stained with Bielschowsky's silver nitrate method. In his preparations he was able to trace the nerve fibres into the dentine. In the lower part of the pulp, fibres occurred which ran transversely between the odontoblasts and the dentine for some distance before entering the dentinal tubules. In the horn of the pulp, the fibres branched into smaller filaments as they ran through the odontoblast layer and then entered the dentinal tubules directly. The author points out that "the microscopic demonstration of the continuity of these fibres involves continual adjustment of the focal plane in accordance with their depth in the section, and it is correspondingly difficult to prepare satisfactory photographs to illustrate their complete course". It is probably for this reason that none of his microphotographs shows the fibres running from the main bundle to the dentine. Neither is it quite clear from his description how far he was able to trace the nerve fibres in the dentinal tubules.

Shortly after Sealey's work appeared, Tiegs (3) published a paper in the *Journal of Anatomy*. He used the same reagents as Sealey but was unable to trace the nerve fibres into the dentine but only as far as the subdentinal space, which he describes as a space between the odontoblasts and the dentine which is traversed by the dentinal fibres. We shall discuss this space further at a later stage in this paper. He unfortunately only published drawings of his

sections, but it is quite obvious from these that he also had seen the two types of nerve fibres described by Sealey.

The discrepancies in the results of these two investigators appeared to justify further work being undertaken on this problem, and it was for this reason that the authors resolved to carry out the research described in this paper. Just as we were completing our work, two papers by Tojoda (4) appeared in the *Deutsche zahnärztliche Wochenschrift*. At an earlier date, this author had claimed to have traced nerve fibres for a considerable distance into the dentine, but his results had never been very convincing. This time, however, his evidence was of a more satisfactory nature and corresponded very closely with the results of Sealey, of which he apparently had no knowledge. He used a very elaborate technique for the preparation of his specimens which is fully described in his paper and would take up too much space here. Essentially, however, it consisted of fixation of small pieces of the tooth in formol followed by decalcification in formic acid. The teeth were then placed in lithium carbonate and after washing were placed in distilled water and submitted to the influence of an electric current with a view to withdrawing from the teeth the products of decalcification. He then used various silver and gold impregnation methods to stain the teeth. Tojoda adopts a rather elaborate system of classification of nerve fibres but essentially they fall into two groups: (1) fibres which enter the mouths of the dentinal tubules and run outwards as intratubercular nerve fibres, (2) fibres which enter the ground substance of the odontogenetic zone and, after running in for some distance, finally pierce the walls of the dentinal canals and then continue as intratubercular nerve fibres. In a very recent paper, van der Sprenkel (2) states that he has been able to trace Tojoda's first type of fibre into the distal parts of the tubules. He also states, however, that he seriously doubts the existence of the second group of Tojoda's fibres, as it would be extremely unlikely that unmyelinated nerve fibres would run for any considerable distance in either the calcified or uncalcified ground substance. He has been unable to find any such fibres.

In our own investigation we employed the standard Cajal technique of fixation with ammoniated alcohol, decalcification in nitric acid followed by silver nitrate and reduction with pyrogallic acid. We also used the Bielschowsky method but never had much success with it. We also employed Tojoda's technique, but the results we obtained were never sufficient to justify the extra amount of labour which their preparation required.

After the specimens had been impregnated, they were cut on a freezing microtome, dehydrated, cleared and mounted in canada balsam.

RESULTS

The nerve bundle after entering the apical canal divides into a number of small bundles which run towards the crown of the tooth, parallel to its long axis. The bundles which lie nearest the dentine supply the proximal part of

the dental tissues, and their place is then taken by the more centrally lying fasciculi which are distributed to the distal portions of the tooth.

As the bundles are traced towards their terminations, they give off fibres which turn into the odontoblast layer. These fibres do not appear to unite or to communicate with each other in any way, and we have been unable to discover anything resembling Raschkow's plexus. On entering the odontoblast layer, the nerve fibres were found either to divide up in a dichotomous manner or pass without any branching into the odontogenetic zone of the dentine.

The distribution of a typical branching fibre is shown in Plate I, fig. 1. This is a drawing which has been prepared for us by Miss Davison and shows the precise course of the fibres. It is to be regretted that it has not been possible to illustrate this with a microphotograph, but on account of the undulating course of the nerve, we found, as other workers have done, that it was impossible under the microscope to obtain a sufficient length of fibre in focus in any one plane to give an adequate idea of its distribution. It will be observed that the nerve gives off branches which run outwards through the odontoblasts and that these undergo dichotomous division and that the terminal branches end in the mouths of the dentinal canals. Although this diagram does not illustrate the point, it has been found possible to trace the nerve fibres for some distance into the tubules. Although we have been unable to find any end-organs such as those described by Tiegs, we believe that many of the branches from these nerves probably end around the odontoblast cells.

The second type of unbranched fibre is illustrated in Plate I, figs. 2 *a*, 2 *b*, and Plate II, fig. 1. Plate I, figs. 2 *a* and 2 *b* are microphotographs of the same field in two slightly different planes. By this means it is possible to trace one nerve fibre from the origin in one of the bundles and to follow its course as it turns outwards through the odontoblasts into the odontogenetic layer of the dentine. Plate II, fig. 1, shows a higher power magnification of the same section. From these three diagrams it will be observed that the second type of fibre is much thicker than the one already described, and that it passes through the odontoblast layer without giving off any branches before it passes into the dentine. In the dentine it has no relationship with the dentinal canals but has a course independent of them. Having thus established that this type of fibre is of nervous origin, we were then able to return to some of our earlier specimens. In these we had seen fibres which resembled very closely those described by Tojoda, and as we had been unable to establish any connection between these and the nerve fibres of the pulp, we considered it necessary, at least temporarily, to reject them. As these fibres had an identical staining reaction with those described in Plate I, fig. 2 *b*, and Plate II, fig. 1, there is little doubt that they are nerves and that Tojoda was right in coming to this conclusion although it had been impossible to establish their continuity with the nerve fibres. Figs. 2 and 3 in Plate II show examples of this type of fibre. In Plate II, fig. 2, a nerve fibre will be observed running

from the pulp into the odontoblast layer where, unfortunately, during the preparation it has been broken. Directly opposite, in the odontogenetic layer, a structure can be seen which has similar staining reaction, and in the light of the evidence from previous specimens it is quite obvious that the proximal fibre had passed through the odontogenetic layer from the pulp and had been continuous with the structure in the dentine. We can safely say that the fibre passed through the odontoblast layer, entered the odontogenetic zone and then divided into two. These two terminal fibres will now be observed running into the odontogenetic layer at right angles to the dentinal tubules.

In Plate II, fig. 3, the fibre after entering the odontogenetic zone runs peripherally for a short distance and then bends backwards towards the pulp. It does not, however, enter the pulp again. In its course in the odontogenetic zone it will be observed that it breaks up into numerous branches. Neither the main fibre nor the branches run into the dentinal canals. We have been unable to satisfy ourselves as to the manner of their termination, but some of our sections suggested that they might enter the dentinal canals.

We have seen numerous examples of this type of fibre in the odontogenetic layer but have been unable to trace either these or the previously described type of fibre peripherally and have up to the present been unable to follow these fibres into the fully ossified region of the dentine. We are therefore unable to confirm the findings of Tojoda that nerve fibres can be found in the tubules of the true dentine. It is possible that Tojoda's first type do pass to the peripheral end of the tubule, but we do not consider that the evidence which he produced establishes the continuity of the fibres of the pulp with those which he found in the tubules of the fully calcified dentine. In regard to the second type of fibre which runs in the partially calcified ground substance we put forward the suggestion that complete calcification may destroy the nerve fibres, and for this reason they are not found in the dentine proper.

We are able to confirm the findings of Sealey and Tojoda that nerve fibres enter the dentine because we have been able to establish the continuity of the nerve fibres at least in the proximal portion of the dentine with the nerve fibres in the pulp itself. With regard to the findings of Tiegs that the nerve fibres ended in the subdentinal zone, we must confess that we have never seen such a zone, but from his diagrams it would appear that this was really the odontogenetic zone of the dentine, and if this is so, then the results which Tiegs obtained fall fully into harmony with those of other workers.

We have to express our thanks to Prof. Stopford for giving us every facility in carrying out this work. To Prof. Wilkinson we are indebted for the supply of newly extracted teeth. Mr Gooding, the department technician, has, with his usual skill, made the microphotographs.

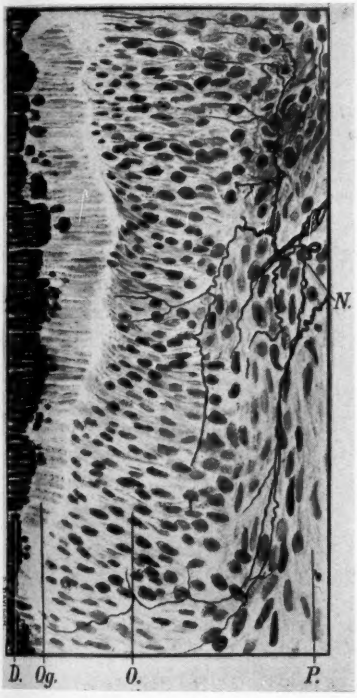

Fig. 1

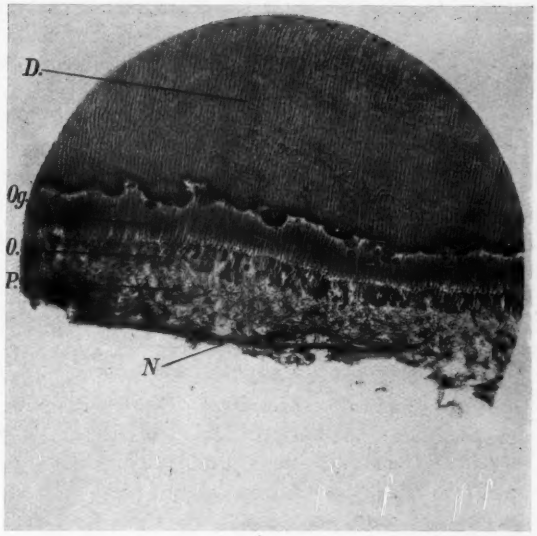

Fig. 2*a*

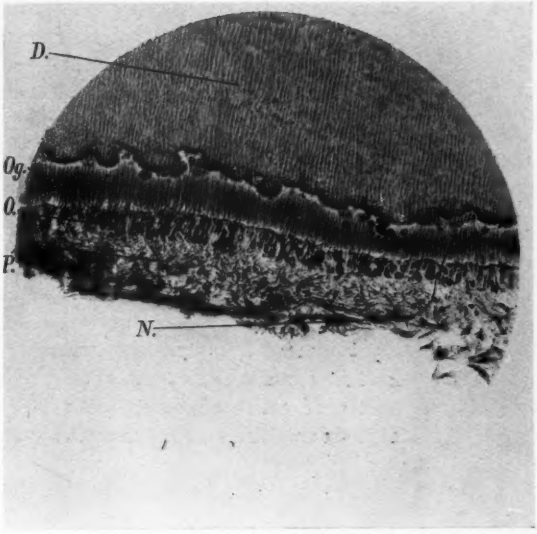

Fig. 2*b*

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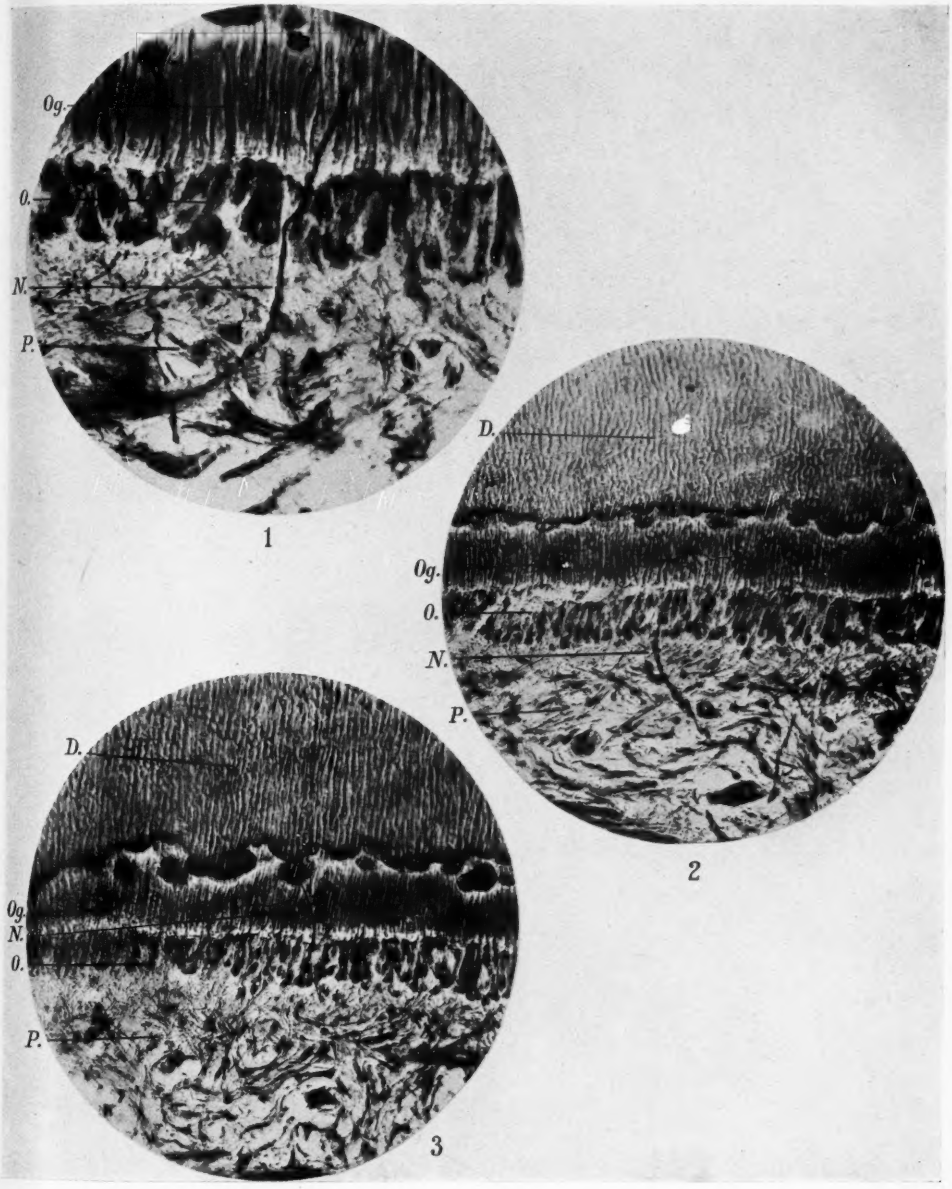

LEWINSKY AND STEWART—THE INNERVATION OF THE DENTINE.

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KEY TO LETTERING ON PLATES

D. Dentine.
N. Nerve fibre.
O. Odontoblast layer.
Og. Odontogenetic layer of dentine.
P. Dentinal pulp.

COMPARATIVE HISTOPHYSIOLOGY OF THE VERTEBRATE NEPHRON

By POL GÉRARD

The Vertebrate kidney is formed of a conglomerate of small tubular units named nephrons (Braus), the function of which is to form the urine. With their distal end, they open into the collecting ducts, which convey the urine towards the urinary bladder.

Two different kinds of nephrons are found among the Vertebrates.

(1) The glomerular nephrons. They are the most common, and usually the only ones the physiologist is dealing with. Their proximal end is dilated

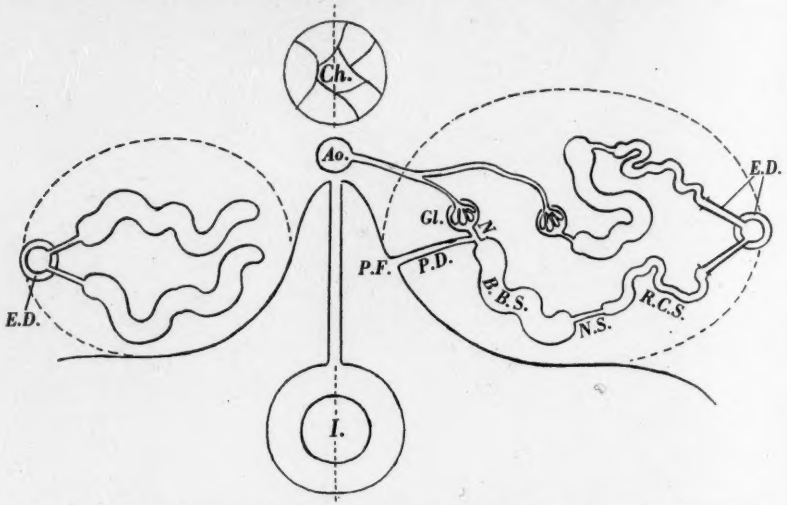

Fig. 1. Schema of the different kinds of nephrons. On the left, the aglomerular nephrons. On the right, the two varieties of glomerular nephrons: below, the open; above, the closed ones. *Ao.* = aorta; *B.B.S.* = brush border segment; *Ch.* = chorda; *E.D.* = excretory ducts; *Gl.* = glomerulus; *I.* = intestine; *N.* = neck; *N.S.* = narrow segment; *P.D.* = peritoneal duct; *P.F.* = peritoneal funnel; *R.C.S.* = rodded cell segment.

in a small ampulla, the glomerular chamber, in which a capillary tuft, the Malpighian glomerulus, hangs by a vascular pedicle; the blood enters the tuft by an artery, and leaves it by another artery which soon opens into an intertubular net of capillaries from which the veins emerge.

(2) The aglomerular nephrons are those the proximal end of which does not present a glomerulus. They are far less common than the glomerular ones; till now, they have been described in some marine Teleosts only (fig. 1).

Amongst the glomerular nephrons we can distinguish two varieties: the open and the closed ones. The former are characterised by the fact that the glomerular chamber, or the neck which connects it with the proximal end of the tube, presents a diverticulum which opens into the splanchnocoele by a wide opening or by a ciliated duct, the latter expanding to form a funnel-shaped orifice as it opens into the peritoneal cavity. Through the peritoneal canal, the nephron and the collecting duct, the body cavity communicates with the external world. On the contrary, the closed nephrons do not possess a peritoneal canal. Usually the glomerular nephron (closed or open) is divided into the following portions: the glomerulus, the neck, the proximal convoluted tube, the narrow segment (which is not constant) and the distal convoluted tube. These names, taken from mammalian anatomy, are somewhat inadequate. The proximal convoluted tube, for example, is sometimes straight and not convoluted. Therefore it seems advisable to rename these portions according to their histological structure. The suggested names would be as follows: glomerulus, neck, brush border (or striated border) segment, narrow segment, and rodded cell segment (fig. 1). As for the aglomerular nephron, it has, in its whole length, the structure of a brush border segment.

The histological structure of these segments is too well known to be described in detail. Nevertheless, it may be mentioned that the cells of the brush border segment contain, regularly in the Poikilotherms and rarely in the Homoiotherms, yellowish granules of varied size, spoken of as secretory granules. As the neck and the narrow segment may be absent, the glomerular nephron comprises three main segments: the glomerulus, the brush border segment, and the rodded cell segment.

Glomerular and aglomerular nephrons are homologous, and originate in the same embryological "Anlage". As the latter are derived from the former, and are strictly limited to certain marine Teleosts, it is logical to take up first the study of glomerular nephrons.

The histophysiology of the nephrons will be first considered, and then subsequently two processes which are intimately connected in the renal function, viz. the glomerular permeability and the storage phenomena in the brush border cells, known under the name of athrocytosis, will be discussed.

I. GENERAL VIEW OF THE HISTOPHYSIOLOGY OF THE NEPHRON

There are two theories of urinary secretion.

The Bowman-Heidenhain theory asserts that the water and the salts are filtered along the glomerulus, while "the tube" secretes into its lumen the characteristic substances of the urine (uric acid, urea, creatinine, etc.).

The Ludwig-Cushny theory admits that through the glomerulus are filtered not only all the components of the normal urine, but some others also, which are dissolved in the blood plasma. This glomerular urine is in a state of high

dilution, and when it passes through the tube it is concentrated by resorption of water and its composition changed through resorption of some of the components of the glomerular urine.

These two theories have one point in common, viz. the filtration of water through the glomerulus. They differ from each other, however, on the composition of the glomerular filtrate, and on the tubular function.

A. Function of the glomerulus

In the year 1878 Nussbaum (33, 34) succeeded in dissociating in the frog the glomerular and the tubular functions by ligation of the renal arteries. In this animal, as in the other Batrachians, the kidney is irrigated by two systems of afferent vessels. The renal arteries form the first one; they give off exclusively the afferent arteries to the glomeruli from which the efferent vessel branches soon into an intertubular capillary net; the second system is represented by the portal vein of the kidney, which runs along its external side, giving off numerous transverse branches which soon pass into the intertubular capillary net: so that in this net runs a mixture of arterial and venous bloods, which finally collects in the renal veins. Taking advantage of this anatomical position, Nussbaum tied the renal arteries. The circulation ceases in the glomeruli, and these are put out of function. The tubular system keeps its own blood supply through the portal vein. Immediately after the operation, the urinary flow stops nearly completely. One conclusion only may be drawn from this experiment: most of the urinary water comes from the glomerulus.

Since then, Wearn and Richards (39, 40), White and Schmidt (41) have succeeded in micropipetting the glomerular space, and collecting the glomerular fluid. In *Necturus*, the glomerular flow amounts to 0·1 mm.^{3} in the space of 10–15 min.

It is thus proved that much water passes through the glomerulus.

Wearn and Richards demonstrated also that the glomerular urine is an ultrafiltrate: it does not contain any proteids; but numerous normal urinary components can be detected in it, as chlorides, urea, uric acid, phosphates; the concentration of the two latter substances is equal to that in the blood plasma (Bordley and Richards (3); Walker, Ellinwood and Reisinger (38)).

When certain dyes are injected into the blood, they are partly excreted with the urine; but it is impossible to ascertain by direct exploration through which portion of the nephron (glomerulus or tube) they are passed. With the micropipette method it has been possible to ascertain that the following dyes are filtered through the glomerulus: methylene blue, phenol red, indigo carmine, which are not colloidal (Wearn and Richards); also ammonium carminate, trypan blue, which are colloidal dyes (Hayman and Richards (24)). Their extreme dilution in the glomerular urine, the narrowness of the glomerular space, and the slight opacity of the interposed membranes prevented the recognition of their presence in the glomerular urine by direct investigation.

Thanks to a method devised by Ellinger and Hirt (12, 13), it is now possible to follow the elimination of an injected fluorescent dye in a normally irrigated kidney when illuminated with ultra-violet light. The slightest traces of the dye become fluorescent and can be easily detected. By this means Ellinger and Hirt succeeded in demonstrating that the fluorescine passes through the glomerulus in both Amphibians and in Mammals. In the frog, Singer obtained similar results with aesculin.

But the glomerular liquid contains substances which are not found in the vesical urine. Wearn and Richards have demonstrated with the micropipette method that glucose is a normal component of glomerular urine. According to Walker, Ellinwood and Reisinger, it amounts to 95 per cent. of the blood glucose. There is no doubt that most of the substances dissolved in the blood plasma are filtered—or, better, ultrafiltered.

These experiments, however, do not exclude the possibility of the same substances being at the same time secreted through the tubule. We are thus led to examine with close attention the tubular function, and particularly that of the brush border segment.

B. Functions of tubules

(*a*) *Secretion*

In favour of the secretory function of the brush border segment, the following arguments have been put forward.

(1) In the cells of the striated border segment of many Poikilotherms and of some Mammals, numerous granules can be seen; they are yellowish, variously sized, and from their similarity to the secretory granules of the glandular epitheliums are known as secretory granules. Some colloidal dyes also appear in these same cells in a granular form, and this has been interpreted as a secretion process. Now, these conclusions are based on wholly superficial resemblance. We shall see later that yellow granules and coloured granules are witnesses of a secretory function.

(2) During these last years, American physiologists (Edwards (8, 9), Marshall (30, 31), etc.) have studied in some detail the urinary function in aglomerular Fishes, the nephron of which is composed only of a brush border segment. In these Fishes, the quantity of urine seems to be slightly inferior to that of the glomerular ones: *Myoxocephalus* (glomerular) supplies 4 c.c. of urine per kg. per day; *Opsanus* (aglomerular) 2·5 c.c. per kg. per day (Grafflin (21, 22)). But in both the components are the same, and in about the same proportions; measurements made on creatine, creatinine, uric acid and sulphates (Marshall (30) and Grollman (23)). The aglomerular kidneys excrete fairly well indigo sulphate and phenol red, this latter in a higher concentration than in the blood. All these substances must necessarily pass through the tubule.

Now, are we allowed to extend to the glomerular nephrons the information gained from the aglomerular kidney, and to infer that their brush border

segment secretes the urinary substances? This would not be justifiable. With the micropipette method, the filtering function of the glomerulus has been demonstrated. The aglomerular structure is a secondary state, which sometimes develops late (Grafflin). It might be possible then that the atrophy—or the non-development—of the glomerulus awakes or increases the secretory capacities of the tubule. Therefore, the physiological features of the aglomerular nephrons must be considered as rather exceptional, and cannot be invoked in favour of the secretory power of the segment in the glomerular nephrons.

So far the evidence for the secretory power of the striated border segment is of little value. This problem cannot really be solved unless the glomerular can be experimentally dissociated from the tubular functions.

To Heidenhain and Neisser (26) must be ascribed the first experimental research in this direction. After sectioning the spinal cord in the cervical region in a rabbit (operation which is followed by a fall of the blood pressure and a nearly complete cessation of the urinary flow), indigo sulphate is injected into the veins, and some minutes later the kidney fixed with a fluid which precipitates the dye. In the sections, a coloured precipitate is observed in the lumen of the tube, and none in the glomerular space, while in the cells of the striated border segment appears a diffuse coloration. For a long time this experiment seemed convincing. Nowadays it is no longer so, for the following reasons: firstly, the circulation is not entirely suppressed in the glomerulus, and it might be supposed that a small quantity of the dye has been passed through it, and has been concentrated as soon as it reaches the tubule.

Secondly, as for the dye found in the cells, close examination shows that only the nuclei are tinged, while the cytoplasm is colourless. This fact proves that diffusion of the dye has taken place, leading to its adsorption on previously coagulated cellular organites.

This experiment was repeated by Nussbaum, in a far more satisfactory way, on frogs with suppressed renal arteries. In these conditions, the indigo sulphate is found as a precipitate in the lumen of the tubes, and as a diffuse coloration in the cells of the segment. This experiment is much more convincing than that of Heidenhain, and in these last years it has been confirmed by Bensley and Steen (2) in the living kidney. After having suppressed the arterial blood supply, these authors examined by direct observation the elimination of the indigo sulphate. Soon after the injection, the dye is detected in the lumen of the brush border segment, and a very slow flow of coloured to tubular urine can be observed.

The phenol red gives the same results, and, moreover, here the dye is more concentrated in the lumen than in the blood plasma.

Ellinger and Hirt were able with their method to follow the elimination of the fluorescine in the kidney of a rat after section of the cervical spinal medulla. The blood flow ceases in the glomeruli. Then fluorescine is injected into the blood, and no fluorescence can be detected in the glomerular space,

nor in the lumen of the tube, but the cells of the striated border segment become slightly fluorescent.

After a subsequent injection of urea, no change is observed in the glomerular space, but fluorescent liquid appears in the lumen of the striated segment, and coloured urine collects in the bladder.[1]

In the Amphibian as in the Mammal, the secretory power of the brush border segment is thus demonstrated.

(*b*) *Resorption*

In the front rank we find the experiments of Richards and his school. We have already seen that Wearn and Richards demonstrated in the Amphibian the presence of glucose and sodium chloride in the glomerular fluid. Now, the vesical urine is always devoid of glucose, and in winter of sodium chloride. These two substances must then have been reabsorbed during their passage through the tubule.

White and Schmidt showed, by micropipetting at more and more distal points of the brush border segment, that the glucose resorption takes place in the first third of this segment. They showed also that the glomerular fluid, which is nearly isotonic with blood serum, becomes highly hypotonic when it has run past this first third.

On the other hand, Hayman and Richards succeeded in introducing with the micropipette some dyes into the glomerular space: methylene blue, phenol red, trypan blue, and ammonium carmine. All these substances (which filter through the glomerulus) are absorbed by the cells of the striated segment where they can be detected *in vivo* or after fixation: but this is a "partial argument", for, as the authors point out, it ought to be proved that the same substances are not secreted through the segment.

Von Möllendorff (37) seems to have found convincing proofs of the resorption of certain dyes (colloidal acid) by the brush border segment.

When such a substance, trypan blue for instance, is injected it is eliminated in part through the kidney. At the same time, the cells of the brush border segment fill with coloured granules.

This storage of the dye presents two peculiarities: there is no concordance between the moment of the maximal dye concentration in the vesicular urine and the moment of the maximal storage in the cells. The dye concentration in the urine reaches its maximal height a few hours after injection; the maximum of storage is obtained after a few days, and will remain unchanged during a long time, while the urine contains but traces of the colour. If the dye were secreted by the tube, no such discrepancy between the curves of the elimination and of the storage would occur.

[1] These results are in contradiction with those of Ghiron; but this author could observe the tubes only and not the glomeruli, and admitted that these latter had recovered their filtering power after the urea injection.

Moreover, when the topographic distribution of the dye in the brush border segment is studied, it appears that the storage is the densest at the initial part of the segment; from this point it decreases gradually, and ceases about the middle part of the segment. This phenomenon may be easily explained in this way. The dye is filtered through the glomerulus in a diluted state (this we know by the micropipetting experiment). As it progresses through the striated border segment, the glomerular fluid is cleared up through the absorption of its coloured particles; and at a certain level, the concentration of the dye becomes so low that no storage phenomena follow its resorption phenomena. Von Möllendorff has thoroughly studied the way by which these storage granula are formed and has come to the conclusion that their formation is the same in the kidney as in the reticulo-endothelial system of Vertebrates. Before him this process had been described by Burian in the Invertebrates under the name of athrocytosis. We will make use of this convenient word to indicate the intracellular flocculation which follows the absorption of electro-negative colloidal particles.

Experiments undertaken by Dawson (6, 7) on *Necturus* afford supplementary proof of the resorption in the brush border segment. In this animal—as in all the Urodeles—the lumbar kidney is composed of a mixture of open and closed glomerular nephrons. After subcutaneous injections of trypan blue, an athrocytosis of the dye develops, with the same intensity, in the two kinds of nephrons.

But if intraperitoneal injections be made, the athrocytosis is much more developed in the open nephrons than in the closed ones (fig. 2, left).

This difference must be ascribed to the fact that the striated border segment of the closed nephrons receives only the dye which has filtered through the glomerulus, while it in the open ones in addition to the same glomerular fluid receives the much more concentrated solution entering through the peritoneal canals. No wonder then that the athrocytosis is by far more conspicuous in the open nephrons. But the athrocytic power of this segment is not restricted to particles which can pass through the glomerular barrier: it is much more extensive, as Lambert (27, 28), Gérard and Cordier (17, 19) have shown, in the Urodeles and the Discoglossidae tadpoles. When solutions of low dispersion (as soluble Prussian blue, or colloidal thorium oxide, or Indian ink Pelikan), the particles of which are unable to pass through the glomerulus, are injected into the peritoneal cavity, athrocytosis takes place in the open nephrons only (fig. 2, right). But, if previously the peritoneal funnels have been experimentally occluded, no athrocytosis at all occurs.

Therefore, this phenomenon depends on the fact that the particles come in close contact with the apical surface of the brush border cells.

It was then interesting to inquire if particles larger than those of Indian ink would also be absorbed by the cells of the segment, and to determine the limit at which this phenomenon ceases.

This we have done by injecting in the same Amphibians a suspension of

minute crystals of cinnabar, or of sepia ink (which is a suspension of very small melanin granules). In both cases these visible granules were absorbed and stored in the cells of the brush border segment, not in its initial portion, but in its distal one. This process can no more be called athrocytosis but phagocytosis. This phagocytosis is restricted to particles smaller than 1 μ, for carbon dust of this size is left unabsorbed.

Thus we may consider the cells of the brush border segment not only as athrocytic, but also as phagocytic cells: they are athrophagocytes in the sense used by Cuenot, with the restriction that they are polarised athrophagocytes, which can exert this power through their apical surface only.

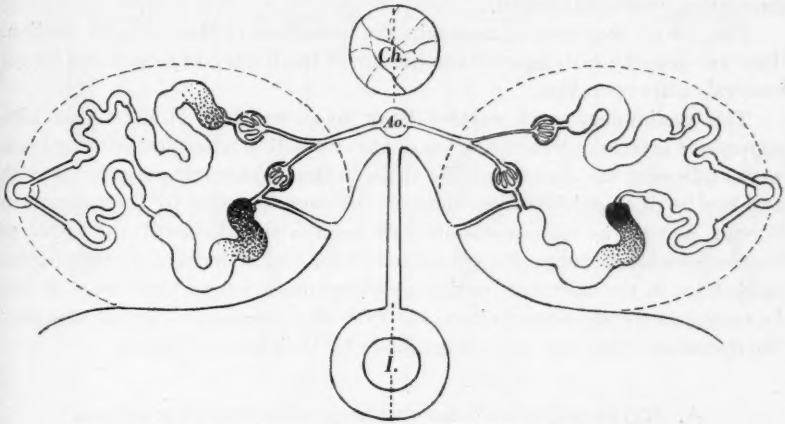

Fig. 2. Schema of the lumbar kidney in an Urodele. On the left, results of Dawson after intraperitoneal injection of trypan blue—athrocytosis much more developed in the open nephrons than in the closed ones. (When the dye is injected subcutaneously, the amount of athrocytosis is equal in both nephrons.) Note the proximally located athrocytosis. On the right, results of Gérard and Cordier, and Lambert, after intraperitoneal injection of Prussian blue, or Indian ink. Athrocytosis takes place in the open nephrons only, and is distally shifted. The same result is obtained after injection of a suspension of melanine granules, or of cinnabar crystals (phagocytosis).

The arguments in favour of the resorbing activity of the brush border segment are thus as impressive as those in favour of its secretion power.

It would then seem quite logical to ascribe to it a double function of secretion and of resorption. But it seems that this idea is regarded as nonsense by most of the experimentalists.[1]

This must be ascribed to the fact that most of the researches have been undertaken with the view of confirming or upsetting one of the two theories.

Gérard and Cordier (15) examined the problem again, restricting the experiments to one species, in which it was possible to dissociate the glomerular

[1] Ellinger and Hirt appear to be the only ones who, after experimentation, support the double function of the brush border segment.

from the tubular function. In this manner they could get results comparable with one another.

After preliminary trials they chose the toad (*Bufo vulgaris, B. calamita*) to experiment on. This Anuran is much better suited to experimental work than the frog, because the suppression of the arterial blood supply in its kidney does not provoke the desquamation of the tubular epithelium. They sectioned first, with the thermocautery, the renal arteries of one-half of the organ (there are four to six renal arteries). As there are no anastomoses between the different arterial territories, the arterial blood supply is abolished in the operated part; the other half of the kidney, which keeps its normal circulation, serves as control.

First of all they had to ascertain the behaviour of the operated portion. This was done by histological examination of the kidney at longer and longer intervals after operation.

This preliminary work enabled them to affirm that, in the region with suppressed arteries, no recuperation of the circulation takes place during three weeks following the operation. The tubes in that region remain quite normal, and can be distinguished from those of the control region by their shrunken lumen. As soon as the circulation is restored in the glomeruli, the lumen of the corresponding tubes widens immediately. The soundness of the tubular epithelium in the operated portion may last much longer than three weeks. In many cases some glomeruli are still without a circulation two months after the operation. They are only recognisable by their narrow lumen.

A. Experiments proving the secretory function of the striated border segment

(1) 1–2 c.c. of a 1 per cent. methylene blue solution is given subcutaneously to an operated toad. When the elimination of the dye is in full progress, a fixing fluid, which acts also as a precipitant towards the colour, is injected through the aorta. By this means the fixing fluid reaches the cells immediately, the shifting of the dye is avoided, and it is precipitated in the vacuoles in which it has segregated. In the sections the dye can be demonstrated in the striated border cells of the operated as well as in those of the normal part of the kidney; it is even more abundant in the former. This fact, which had been observed previously *in vivo* by Ellinger, can be explained by a delayed transit of the dye through the cells of the operated region (fig. 3).

(2) Under the same experimental conditions, uric acid previously dissolved by addition of piperazine (2 injections of 0·05 gm. of uric acid at 20 min. interval) is injected, and, half an hour after the last injection the kidney is fixed by an intra-aortic injection of the van Gehuchten fluid, in which urate of piperazine is insoluble. Thanks to its low diffusibility this salt is precipitated without shifting, and its localisation can be traced with the polarisation microscope.

In the normal region of the kidney crystals are detected in the cells of the brush border segment and more abundantly in its lumen. Occasionally a crystal may be discovered in the glomerular space. In the operated region most of the crystals are included in the cells of the same segment, and few in its lumen. On the contrary in both regions of the kidney the cells of the rodded segment or those of the collecting tubules appear quite free from crystals (fig. 3).

Thus the brush border segment is able, alone, to eliminate methylene blue and uric acid: it behaves as a secretory organ.

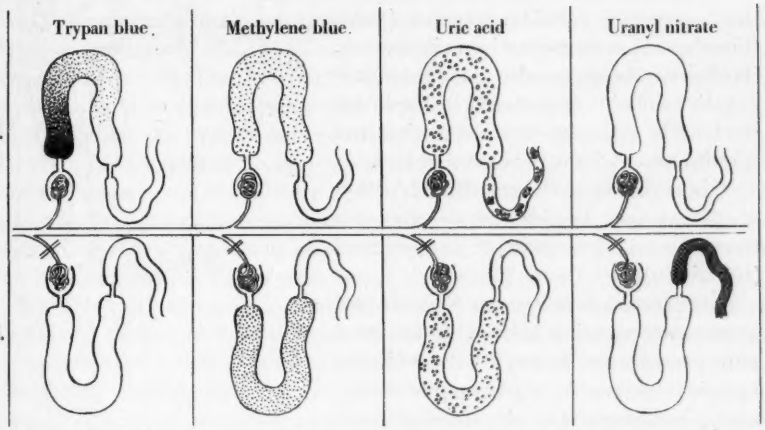

Fig. 3. Schema of the experiments of Gérard and Cordier on the toad. The upper part represents the normal nephron (*N.*), the lower one, the nephron with the glomerular blood supply suppressed (*S.*) after injection of various substances.

B. Experiments proving the resorbing function of the striated border segment

Quite different are the results obtained after injections of a colloidal electronegative dye, as trypan blue or ammonium carminate. In these instances, the dye is only found in the normal portion of the kidney, where it has been stored with a maximal athrocytosis at the beginning of the brush border segment. On the contrary, in the operated part, all the cells of the segment are empty.

Thus, the brush border segment behaves quite differently towards a non-colloidal basic and a colloidal acid dye. It can absorb the first one through its basal surface, which proves quite impermeable for the second one; on the contrary, the same acid dye is absorbed and stored when it comes in contact with its apical surface (fig. 3). Moreover, there is a parallelism between the intensity of the glomerular circulation and the extent of the athrocytosis. This is rendered evident when injecting toads more than three weeks after

operation. At that time, there exists in the operated region of the kidney a mixture of glomeruli which have recovered their normal circulation, of others which remain streamless, and lastly of others in which the blood supply is still small (this is detected by the slight quantity of Indian ink sticking to the capillar endothelium). The brush border tubules related to these three species of glomeruli exhibit a quite different degree of athrocytosis: in the first tubules it is as developed as in the normal region; in the second there is none; in the third it is but poorly apparent.

The same results are obtained with other electro-negative colloids: solganal (aurothio-glucose), ammonium iron citrate, etc. Ovalbumin behaves also as an electro-negative colloid: it is stored in the brush border cells of the non-operated region, and is quite absent from the operated one.

These proofs of the resorbing function of the brush border tubule are also strengthened by another experiment (Gérard (14)).

The Teleost *Lepadogaster Gouanii* possesses a very peculiar kidney. In the front part persists the pronephros constituted by a single glomerular nephron; posteriorly, a set of mesonephric lobules are found, each of which is formed by some aglomerular nephrons. If trypan blue be injected into this fish, the dye is found stored in the striated border segment of the pronephros, the athrocytosis being there at its height in the initial part. On the contrary, no dye can be found in the mesonephros. Now, the two species of nephrons differ from each other by the glomerulus only. It must then be concluded that the athrocytosis in the pronephric nephron originates in the resorption of the dye which has been passed through the glomerulus (fig. 4).

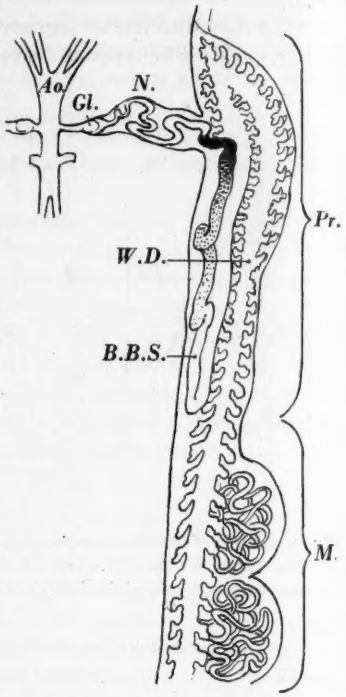

Fig. 4. Schema of the anterior part of the kidney of *Lepadogaster Gouanii* after an injection of trypan blue. Athrocytosis takes place in the brush border segment of the glomerular nephron only. *Ao.* = aorta; *B.B.S.* = brush border segment, showing initial athrocytosis; *W.D.* = Wolffian duct; *Gl.* = glomerulus; *M.* = mesonephros; *N.* = neck; *Pr.* = pronephros.

Summing up all the experimental data, these two conclusions may be drawn: through its basal membrane the cell of the striated border segment acts as a glandular cell: it is secretory. Through its apical membrane it resorbs some of the plasmatic substances which have filtered through the glomerulus—crystalloids and highly dispersed colloids—the resorption of these latter being

followed by their athrocytosis. In the case of open nephrons, resorption and athrocytosis of much bigger particles takes place.

C. Function of the rodded cells segment

All the histophysiologists agree to ascribe to this segment a water-resorbing function. When a living kidney is examined while excreting a dye, it can be noticed at first sight that the coloration of the liquid flowing in the lumens is much more marked in the rodded cell segment than in the striated border tubule. Never has there been a secretory process demonstrated in it. On the contrary, we have obtained numerous proofs of water resorption at its level.

(1) Bensley and Steen observe *in vivo* the elimination of indigo sulphate through functionally isolated tubules. The urinary flow, of course, had become very slow. When the fluid reaches the rodded cell segment, crystalline deposits, formed by minute needles of the dye, appear in the lumen.

(2) During the experiments on the elimination of uric acid, it is always observed, in the normal portion of the kidney, that the lumen of the brush border segment contains a very fine crystalline dust, while in that of the rodded cells segment there appear very large birefringent deposits. As no secretion is conspicuous in this latter segment, this picture shows evidently that the uric acid has been concentrated in the rodded cell segments.

(3) If uranyl nitrate be injected into a toad, this salt is eliminated through the kidney. It occasions a necrosis in the rodded cell segment where it can be easily detected.[1] It is quite impossible, with this sole experiment, to discover the mechanism of the lesions. But if we repeat it after partial suppression of the renal arteries, no injury at all can be discovered in the operated portion of the kidney. The suppression of the glomerular function has protected the kidney against the nocuous action of the uranyl salt. Thus, this substance is eliminated through the glomerulus and passes through the brush border segment without causing any injury because of its insufficient concentration; but as soon as it reaches the rodded cell segment, its concentration increases to such a degree, through resorption of water, that necrosis follows (fig. 3). Thus, to each segment of the glomerular nephron may be ascribed a different function. The glomerulus acts as an ultrafilter; the glomerular urine which it passes undergoes quantitative and qualitative changes during its course through the brush border segment, by a double process of secretion and resorption. Finally, as it reaches the rodded cells segment, it concentrates through water resorption.

II. The athrophagocytic function of the nephron and its significance

One of the most astounding features of the brush border segment is the storage process of colloidal substances known as athrocytosis. From this standpoint, a difference of behaviour exists between the closed and the open

[1] In the Mammals, the lesions are located in the striated border segment.

nephrons. In the former indeed are only stored the micellae which can pass through the glomerular barrier; the second are capable of exerting their athrocytic function on much larger particles. This difference must be ascribed to the presence, in the open nephrons, of the peritoneal duct, and not to a difference in the glomerular permeability.

Now if we examine the localisation of the athrocytosis all along the brush border segment, we are struck by the fact that it is not the same for the small or for the large micellae. Von Möllendorff had already noticed that the athrocytosis of the trypan blue is localised at the proximal end of the brush border segment, while that of the less diffusible pyrrol blue is shifted somewhat distally. From this observation he had drawn the conclusion that the distal portions of the brush border segment are more permeable than the proximal ones, since they can reabsorb bigger particles. But exact data concerning the dispersity of colloidal solutions were not available then, and without these it was impossible to attempt accurate investigations.

Thanks to a better instrumental equipment, Gérard and Cordier (17,18,19) were able to undertake the study of this problem in the Anurans and some other Vertebrates, while Lambert (28) did the same for the Urodeles. The starting-point of our researches consists in an observation made after an intramuscular injection of tellurium in the toad. It had been shown by Levaditi that if a suspension of tellurium be injected intramuscularly, it is resorbed and eliminated partially through the kidney, in the brush border segments of which the metalloid appears as a deposit of black granules. This experiment was repeated in the toad with the same results, and we started to inquire through what portion—glomerular or brush border tube—tellurium was eliminated. Having injected the metalloid into toads whose renal arteries had been partially suppressed, we found the telluric granules only in the normal portion of the kidney; in consequence they must have been filtered through the glomerulus, reabsorbed and stored into the cells of the striated border segment. Moreover, the athrocytosis of the tellurium does not begin immediately after the neck, as it does for trypan blue; but it has shifted distally, and its maximum density occupies the region of the yellow granules. This can be better demonstrated on operated toads which have been injected successively with tellurium and ammonium carminate: the initial portion of the brush border segment presents thus a very dense athrocytosis of the red dye, which decreases gradually; then comes an intermediate portion where scarce granules of carmine and of tellurium are found together; more distally, telluric granules only are conspicuous (fig. 5). This experience demonstrated that the striated border tube behaves, in its storage of substances filtered through the glomerulus, as if it were composed of portions performing each a different function; but it was then impossible to give a satisfactory explanation of this phenomenon.

When Lambert's researches had shown that the Indian ink was stored by the striated border segment in open nephrons (which was confirmed afterwards

by Dawson), we decided to make use of the open pro- and mesonephric nephrons in the tadpoles of *Rana*, *Discoglossus* and *Alytes* in order to investigate the athrocytic localisation of colloids presenting a different degree of dispersion. If we inject into the splanchnocoele of tadpoles a solution of trypan blue or of ammonium carminate, which is able to be filtered through the glomerulus, an athrocytosis of this substance develops in the proximal end of the striated border tube, and presents a maximum of density just at its beginning.

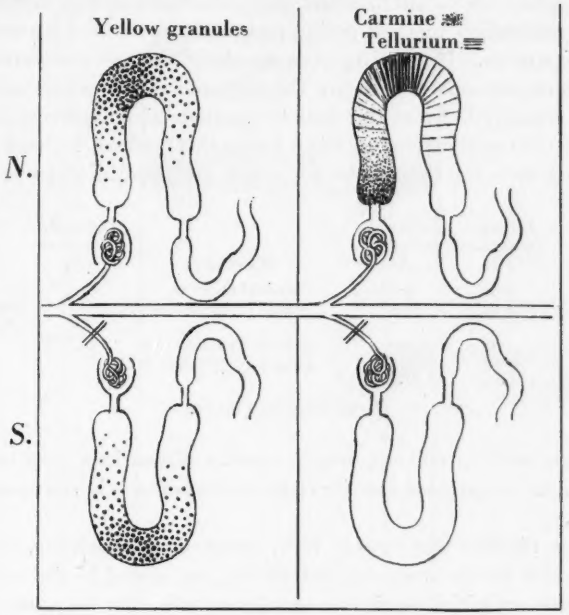

Fig. 5. Schema of the localisation (*a*) of the yellow (so-called secretory) granules, (*b*) of the athrocytosis of carmine and tellurium, in the toad. Above, normal nephron (*N*); below, nephron with suppressed arterial glomerular supply (*S*). The level of the tellurium athrocytosis corresponds to that of the yellow granules. The suppression of the glomerular blood supply does not affect the amount of yellow granules, which form very slowly, and, when stored, remain in the cell.

On the contrary, after an injection of Indian ink, no athrocytosis can be detected in the initial portion of the tube; it appears, but very sparse, near the middle part of the tube, and from this point increases distally till it reaches a maximum density at the end of the segment. Moreover, if ammonium carminate (or trypan blue) and Indian ink are injected on successive days into the same tadpole, the first substance is found stored in the initial, the second one in the distal portion of the striated border tube. And the same result may be obtained if the order of injections be reversed. Here we

find, but with an extremely developed shift, the figures we had obtained in the toad after the double injection of carmine and tellurium. The difference in dispersion between trypan blue (or ammonium carminate) and Indian ink is easy to control by simple methods. Biologically, it can be easily demonstrated by the fact that the first two dyes are filtered through the glomerulus, while the ink is retained by it: but these data are but approximate, and it would have been advisable to get precise measures of their respective dispersions. It would have been interesting also to make use of substances the particles of which are of intermediate size between these two extremes.

The ultrafiltration method giving poor results, we used for this purpose Nistler's apparatus. It consists of a specially devised comparison microscope which enables one to measure the diffusion coefficient in water[1] of the substances employed. From this data the particle dimensions can be deduced with the help of the Einstein formula.[2] Using this method, we have calculated the following data for the substances which give rise to athrocytosis in the kidney:

Substances	Diffusion coefficient (D)	Radius of particles Å.	Substances	Diffusion coefficient (D)	Radius of particles Å.
Trypan blue	3·03	6·5*	Colloidal thorium oxide (Thorotrast)	—	80 (from the von Heyden firm)
Ammonium carminate	1·94	10·2*			
Ink R. A. L.	1·83	10·8	Soluble Prussian blue	0·17	115*
Chlorophyll	0·83	23·3	Indian ink (Pelikan 541)	—	>1000
Iron saccharate	0·76	24·6			

* See footnote 2 below.

With this series of colloids, whose micellar dimensions vary between 6·5 and 1000 Å., we determined the site of their athrocytosis in the open nephrons of tadpoles.

We know already that trypan blue, ammonium carminate, the micellar radius of which lies between 6·5 and 10·8 Å., are stored in the brush border segment, with an initial maximum of athrocytosis. On the other hand, the Indian ink for bacteriology R. A. L. gives also maximal initially located athrocytosis: its micellar radius is 10·8 Å. The athrocytosis of these four substances is equally intense in the closed and in the open nephrons, when injected subcutaneously. When the injections have been given into the splanchnocoele, it appears of course more intense in the open nephrons, which receive, through the peritoneal canal, a supplementary dose of the dye.

After an injection of chlorophyll (23·3 Å.) or of iron saccharate (24·7 Å.) athrocytosis always takes place in the open nephrons: its maximum is no more initial; it has shifted somewhat distally, and is situated near the end of the first quarter of the striated border tube. In the closed nephrons,

[1] Hanut and Fautrez (25) have shown that the diffusion coefficient of the dyes used *in these experiments* is not affected by addition of serum, nor of salts in the same proportion as in the blood.

[2] The radius calculated by this method is but approximate for salts (and dyes behave as salts); it is quite reliable for such substances as chlorophyll, haemoglobin and other proteids.

athrocytosis appears irregularly: present in some animals it is lacking in others. In this case, individual factors have been set in action to prevent or to allow the glomerular filtration of particles of 23–24 Å. Thus this dimension of the particles marks the limits of the glomerular permeability in the Anurans. Above these numbers, the athrocytosis takes place solely in the open nephrons. Likewise thorium oxide, collargol, soluble Prussian blue, the particulate radius of which ranges between 80 and 115 Å., are only stored in the open nephrons,

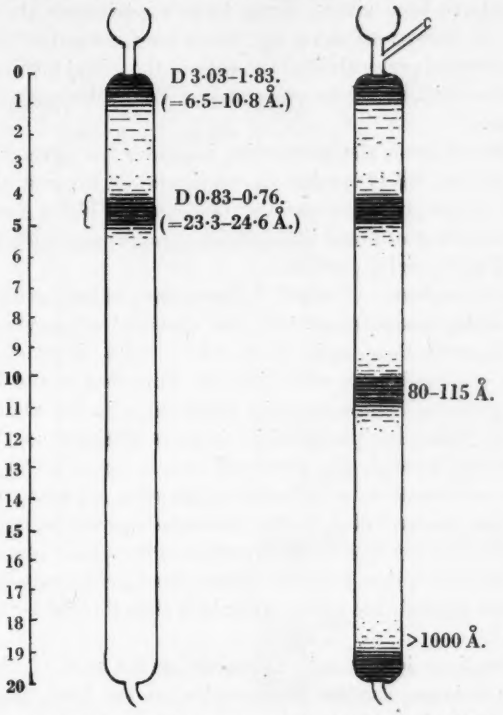

Fig. 6. Schema showing the levels of the maximum of athrocytosis for different substances in the brush border segment of Discoglossidae tadpoles. On the left, closed; on the right, open nephrons. This localisation is the more distally shifted as the diffusion coefficient is low (or as the particle dimension is great).

their maximal athrocytosis being located near the middle portion of the striated border tube (fig. 6).

Finally, at the end of our series, Indian ink Pelikan 541, whose particulate radius measures more than 1000 Å., is stored in the distal portion of the segment, with a terminal maximum of athrocytosis (fig. 6). Moreover, if the same animal be injected successively with solutions differing widely from one another in dispersion, as trypan blue, thorium oxide, Indian ink Pelikan 541, these three substances are received in the brush border segment, each

with a localisation of its own: the trypan blue in the proximal, the thorotrast in the middle, and the ink in the distal portion. In each of these portions athrocytosis reaches its maximum at a very characteristic level: initial in the first one, median in the second, and distal in the third. These portions are separated from each other by transitions in which are found side by side scanty granules of the two adjacent areas.

This athrocytic picture remains unchanged, whatever be the order in which the injections have been given. From these experiments the following conclusions may be drawn: all along the brush border segment the cells do not enjoy the same apical permeability; the cells of the initial portions are pervious to the smallest colloidal particles only, those which, indeed, are filtered through the glomerulus.

As the distance from the glomerulus increases the permeability becomes greater and greater, till it reaches its maximum at the end of the segment. At this level, phagocytic processes also take place. Thus a gradient of apical permeability may be postulated in the striated border segment, which increases from its initial to its distal portion.

In the open nephrons of adult *Salamandra* the general outline of the athrocytosis is the same, except that the maximal athrocytosis of the substances with a particulate radius from 80 to 115 Å. is much more distally shifted, and is distinguishable with difficulty from that of the Pelikan Indian ink. But the gradient is here as easily observed as in the Anurans. Further, the limit of the glomerular permeability is more distinct: so, the chlorophyll (23·3 Å.) is filtered through the glomeruli and is stored in all the nephrons, while the iron saccharate is to be found in the open nephrons only. The limit of the glomerular permeability, in the *Salamandra*, may be fixed at 24 Å. In the closed nephrons, the gradient of permeability exists also, but it is less conspicuous, the resorption and the athrocytosis being only exerted on the particles filtered through the glomerulus: it is thus limited by the glomerular permeability.

But this gradient is not only apparent in the case of electro-negative colloidal dyes; it is as clear for the proteids, as has been demonstrated by Lambert in *Salamandra* (28).

Unhappily, our information about the micellar dimensions of the different proteids is still very scanty and, as they are colourless, it becomes impossible to follow their diffusion speed with the Nistler microscope. But we can draw up a list in which they are arranged according to the increasing order of their molecular weight, and we get the following table:

Substances	Molecular weights	Observations
Ovalbumin	34,000 (after Bayliss, Kerridge and Russell)	—
Haemoglobin	68,000 „	—
Serum albumin	68,000 „	69,000 (after Roche, Dorier and Marquet)
Serum globulin	103,000 „	150,000 (after Roche and Braco)
Casein	788,000 „	—

We already know that in the Anuran kidney injections of ovalbumin, as of coloured electro-negative colloids, are followed by athrocytosis.

How do the Salamander nephrons behave towards these different-sized proteids? After injecting them into the splanchnocoele, all of them are found stored in the striated bordered segment of the open nephrons, but the maximum of athrocytosis has, for each one, a different localisation. In order to locate it accurately, reconstructions must be made from serial sections of the

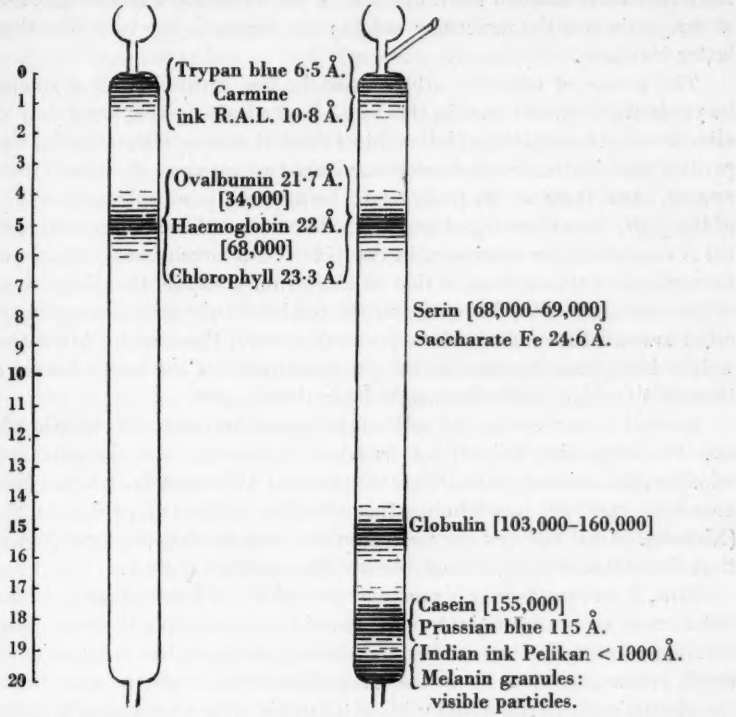

Fig. 7. Schema showing the levels of the maximum of athrocytosis for different substances in the brush border segment of *Salamandra*. On the left, closed; on the right, open nephrons. The localisation of athrocytosis follows the same law as in the Anuran nephrons.

tube. For the sake of an easy study, let us take as reference an uncoiled brush border segment, divided into twentieths, on which these maxima are plotted.

The maximum of athrocytosis, for ovalbumin, is not initial; but it has shifted in the region of the $\frac{4}{20}$th–$\frac{5}{20}$th. Its limits are not sharply marked distally, the ovalbumin producing in that region vacuolisations in the cytoplasm of the segment (fig. 7).

It is impossible to give precise details about the location of the haemoglobin athrocytosis; the histochemical detection of this substance is only practicable

on frozen sections, from which reconstructions of the tube cannot be made; nevertheless it may be suggested that this maximum is located in the first half of the segment.

As for the serum albumin, its athrocytosis is maximal at the level of the $\frac{9}{20}$th; for the serum globulin, between the $\frac{16}{20}$th and $\frac{17}{20}$th; for the casein, in the last twentieths of the brush border tube (fig. 7).

If we compare now these results with the data given in the table, we may infer that there exists a relation between the localisation of the maximum of athrocytosis and the molecular weight: the former is the more distal as the latter increases.

The power of selective athrocytosis in the brush border segment can be yet better demonstrated in this way. Instead of injecting separately serum albumin or serum globulin, let us inject diluted serum. Theoretically, we may predict that in the brush border segments two maxima of athrocytosis will appear. And these we do really find; the first one is conspicuous at the level of the $\frac{7}{20}$th; from there the athrocytosis decreases and then increases gradually till it reaches a new maximum in the $\frac{17}{20}$th of the brush border segment. The first region of athrocytosis is that of the serum albumin, the distal one, that of the serum globulin. Thus, the striate bordered tube acts as a sorting apparatus towards the mixture of the protein micellae; those with a low molecular weight have been resorbed in the proximal parts of the brush border tube; those with a high molecular weight in its distal parts.

Instead of expressing the relation between the maximum of athrocytosis and the molecular weight, let us try if it can be done in terms of the micellar dimensions of the different proteids. Till recently, we had but one measure, that of ovalbumin, the micellar radius of which is 21·7 Å. (Nichols). With Nistler's method, Lambert and Fautrez[29] have just found that the radius of the haemoglobin micella amounts to 22 Å.

Now, if we compare two substances of widely different chemical formula, but of near molecular weight as ovalbumin and chlorophyll, we see that the maximums of their athrocytosis are located nearly at the same point of the brush border segment. From the level of its athrocytosis, we may thus infer the approximate particulate radius of a proteid whose dispersion is unknown. By this means we may estimate the particulate radius of the serum albumin at 22–24 Å. (its maximum of athrocytosis agreeing with that of iron saccharate); the radius of the serum globulin particle must be situated between 40 and 50 Å., since its maximum of athrocytosis is localised before that of thorotrast (80 Å.); and the radius of the casein must be greater yet.

Through an indirect means it is possible to get a more accurate value for the micellar radius of the serum albumin. The researches of Bayliss, Kerridge and Russell[1] have shown that, in the dog, proteins with a molecular weight greater than 68,000 are not eliminated through the kidney, while those with an equal or inferior weight are excreted. But their experiments do not inform us about the site of elimination in the nephrons. We have already seen that

in *Salamandra* the ovalbumin and the haemoglobin are filtered through the glomerulus, and that they are stored in both open and closed nephrons. On the other hand, injections of serum globulin and of casein are followed by athrocytosis in the open nephrons and only after intraperitoneal injections.

As for the serum albumin, subcutaneous injections of this substance are followed by a slight athrocytosis in both nephrons, but only after numerous injections. Thus serum albumin is just at the limit of the glomerular permeability, which can be said to be a little higher in *Salamandra* than in the Mammals. We have found previously that this limit, when expressed in terms of the micellar radius, is 24 Å. Therefore we may fix at 24 Å. the value of the micellar radius of the serum albumin.

In the Vertebrates, other than Urodeles, whose kidney is formed by closed nephrons, athrocytosis is also found in the brush border segment; but it is restricted to those particles which can filter through the glomerulus.

In the Mammals, the glomerular filtration limit is lower than in the Salamander, as no athrocytosis can be obtained by injections of chlorophyll. Moreover, the permeability gradient is more developed in the Mammals, for the athrocytary maximum of trypan blue (6·5 Å. radius) is not localised at the very beginning of the tube, but is shifted a little distally. It may be inferred that in Mammals the apical permeability in the initial cells of the brush border segment is less than in the Poikilotherms, and that in this region are resorbed particles of very minute radius (less than 6·7 Å.), and which therefore do not give rise to athrocytic granules (fig. 8).

It was interesting to inquire about the existence of athrocytosis in the renal organs of Invertebrates. In most of them indeed the urinary organs or nephridia are homologous to the tubular part of the Vertebrate nephron; these nephridia are often closed at their proximal end, like the aglomerular nephrons, and consequently never will exhibit any athrocytosis phenomenon. But some nephridia are open, and, like the open Vertebrate nephron, connect the body cavity with the external world. The most propitious of these open nephridia for experimentation is that of the earthworm. It has a very complicated structure, which we will schematise in this way. It is made of three lobes, joined together at their base, the middle one being the longest. At its beginning, a ciliated nephrostome is differentiated, which is continuous with the nephridial tube; this much-coiled tube runs in a very complicated fashion through the three lobes, and opens at its end into a small urinary bladder. Cytologically, it is differentiated in several portions, one of which, the ciliated segment, presents a structure rather similar to the brush border segment. It runs straightway through the median lobe, and opens into a dilated ampulla. The coelomic liquid is forced through the nephrostome by the long cilia which cover it; after a long run it enters the ciliated segment at the base of the second lobe, and leaves it at its summit, where begins the ampulla; from that point it comes back to the first lobe, and finally passes into the third one, and from it into the bladder.

Cordier (4) has undertaken a series of experiments on this nephridium. After injections of electro-negative colloids into the coelom, athrocytosis, which can never be obtained after injections of non-colloids, occurs in the ciliated segment; here also it presents a maximum, the localisation of which is different according to the injected substances. For colloids, the radius of which ranges from 6·5 Å. to 115 Å., the maximum of athrocytosis is at the initial part of the ciliated segment; from that point it decreases gradually. But if particles of 1000–1200 Å. radius be injected (Indian ink Pelikan), the maximal athrocytosis is shifted distally and located in the middle portions of the ciliated segment[1] (fig. 8).

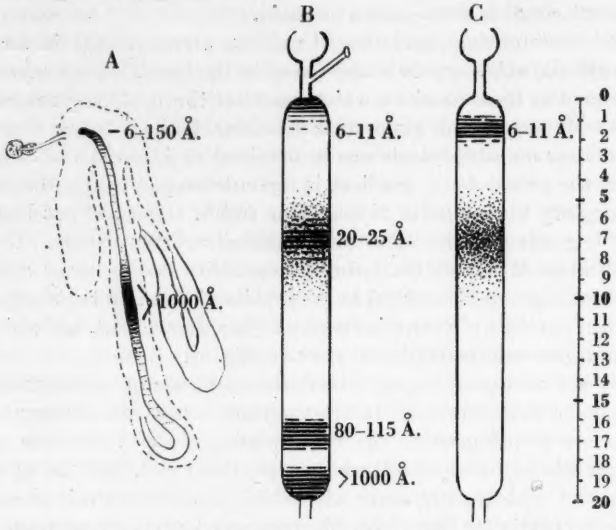

Fig. 8. Schema of the localisation of the maximal athrocytosis. A, in the Oligochaete nephridia; B, in the Urodele nephron; C, in the Mammalian nephron. The stippled areas correspond to the portion filled with yellow granules.

Thus it appears that there exists also an apical permeability gradient in the nephridia as in the Vertebrate nephron. But its value is not the same in the animal kingdom; it varies gradually from the Invertebrate to the Mammal, and is characterised by a progressive diminution of the apical permeability in the initial portion of the tubular system. In the earthworm this initial portion stores particles the radius of which ranges from 6·5 to 115 Å.; in the Amphibians it stores particles from 6·5 to 10·5 Å. radius; in the Mammals it does not allow these same particles to pass. The discriminating power of the athrocytic segment for particles of different size is only slightly indicated in the Oligochaete; it becomes very efficient in Amphibia; it is

[1] In this area also are phagocytosed the small melanin granules of *Sepia*. This portion exhibits an athrophagocytic function similar to that of the distal end in the Vertebrate open nephrons.

highly developed in Mammals. This physiological improvement conforms with the morphological evolution of the animal kingdom.

Thus in the brush border segment of the Vertebrate, as in the ciliated segment of the earthworm, the resorbed substances can be readily recognised by these two characteristics: they are found in a granular form; they are unequally distributed in all the cells of the segment. As granules they are stored in limited portions of the segment, the localisation of their athrocytosis varying with their dimensions. Consequently, whenever a localised region of athrocytosis is located in a nephron, it may be concluded that the stored substance is a colloid which has been reabsorbed by the cells of that part.

Now, in most of the Poikilotherms, a portion of the brush border segment—approximately the second quarter—contains regularly the yellow granules we mentioned before. In the proximal part of the segment these granules are small and scanty; distally they become bigger and more numerous, till a maximum of density is reached, localised roughly near the middle of the segment; from this point their volume and number decrease gradually, and soon the rest of the segment appears quite empty (figs. 5 and 8).

These granules are considered as secretory granules from their likeness to the granules found in the glandular cells. This resemblance is most striking if oblique sections of an Amphibian kidney be examined: here are found side by side sections of the segment in which cells are filled with large granules, while in others the cytoplasm contains only a filamentous chondriom; between these two extremes other sections exhibit all transitional stages. A mere seriation of these pictures would give all the stages of a secretory process as has been done frequently—if longitudinal sections, or teasings of the segments, were not examined: then, the localisation of the granules becomes conspicuous with their maximum of density in its middle part. This is rendered more striking after an injection of trypan blue or ammonium carminate: the athrocytosis of the dye—with its initial maximum—fills the first portion of the segment and then decreases; afterwards comes a region in which coloured and yellow granules are found in the same cells, till a region is reached in which yellow granules only are conspicuous.

Moreover, if the formation of these granules was the result of a cyclic process of secretion it would be logical to expect also the presence of large granules at the beginning or at the end of the supposed secretory region of the tube, and small ones in its centre. Now, this never happens, and the seriation of the granules occurs always in the same fashion. The yellow granules, with their characteristic localisation, have been found in the following families: Cyclostomata, glomerular Teleosts, Ganoids, Dipnoi, Anurans, Chelonia, Ophidians, and Lacertians. Let us point out here the absence of these granules in the aglomerular nephrons, which are purely secretory.

In the homoiothermic Vertebrates, the absence of granules in the brush border segment cells is an established idea. Still, two exceptions have been

pointed out, both concerning hibernating Mammals. Ferrata, in the marmot, and Suzuki, in the hedgehog, have found that during winter the brush border segment cells are filled with yellow granules, without noticing yet any special localisation. In teased preparations from the winter hedgehog kidney, it has been possible to demonstrate that the yellow granules are only present, with a median maximum of density, in the second quarter of the brush border segment. And this can be better illustrated by an injection of trypan blue into the animal: an initial coloured athrocytosis arises, behind which appears the yellow granule region: this figure is similar to that presented by the Poikilotherms.

For all these reasons we may conclude that the yellow granules are not secretory; they are the result of an athrocytosis exerted on colloidal particles which have filtered through the glomerulus, and the size of which is slightly under the filtration limit. When a great number of these granules has been heaped up in the cell, these undergo desquamation, readily followed by necrosis. Thus, athrocytosis is not merely an experimental phenomenon: it is a normal function performed by the kidney in many Vertebrates.

As we have seen, most of the homoiothermic kidneys do not exhibit any athrocytosis. It might then be questioned if their brush border segment has lost this property or if it does not show it for lack of opportunity.

Examples taken from pathology give full answer to this question. We will choose them from the human pathology for two reasons: first, because it is the best known pathology of all homoiotherms; secondly, because in the normal state the human kidney never exhibits athrocytosis.

These pathological conditions are characterised by an increased glomerular permeability: serum albumin which filters through the glomerulus is expelled with the urine, inducing in a limited portion of the brush border segment cytoplasmic changes known as albuminoid degeneration ("Trübe Schwellung" or "albuminöse tropfige Degeneration" of the German pathologists). In that state, the cytoplasm becomes filled with rounded, colourless, refringent granules; they give positive Derrien-Turchini reaction just as the protein granules produced in the Salamander nephron after serum albumin injections.

In another pathological state, the lipoid nephrosis, an increased glomerular permeability develops in a subject affected at the same time with hypercholesterolaemia. Serum albumin and sphero crystals of cholesterol are passed with the urine of these patients, in which they are easily detected.

Now, in the blood plasma, cholesterol is in a colloidal state. In this same state it is filtered through the glomerulus. During its course through the kidney its physical state must have been changed, since it is found crystallised in the urine. Now, if kidney sections coming from a case of lipoid nephrosis be examined, two different kinds of granular formations are discovered in the brush border segments, each one presenting its own and limited localisation. At one level of the tubule, protein granules fill the cells; in another, birefringent crystals or spherical crystals whose cholesterolic constitution may

be ascertained with the Lieberman reaction. Thus two regions of athrocytosis are detected in the same brush border segment (Gérard and Cordier (16)).[1]

If we recollect the experimental results we have obtained, we may explain in this way the successive phenomena which developed in the kidney. Owing to the increased glomerular permeability, superfluous blood cholesterol and serum albumin have been both filtered in a colloidal state through the glomerulus. Their dispersion being different they have been reabsorbed and stored in two different regions of the brush border segments; at the same time cholesterol has crystallised in the cells which subsequently have undergone desquamation when they became overloaded: this explains the occurrence of spherical crystals in the bladder urine.

It is easy to produce the same pictures in the Salamander. After two or three intraperitoneal injections of hypercholesterolised serum, cholesterol and serum proteins are reabsorbed and stored, causing a double athrocytosis in the brush border segment of the open nephrons only.

The same figures can be obtained in the closed nephrons after having increased artificially the glomerular permeability. This can be readily done with injections of ovalbumin: it is known that if ovalbumin is injected into a Mammal, it is filtered through the glomerulus; but, while passing through it, it increases the glomerular permeability to such an extent that serum albumin is also eliminated. Starting from that point, let us inject ovalbumin and hypercholesterolised serum into the body cavity of a toad, the renal arteries of which have been partially ligated. After numerous injections, an athrocytosis of proteins and cholesterol can be observed in the brush border segment of the normal part of the kidney, each one with a special localisation; in the ligated part, on the contrary, no athrocytosis at all can be detected.

Thus the closed nephrons of homoiothermic Vertebrates have preserved an athrocytic power similar to that existing in the open ones. Under normal conditions (beside the hibernating Mammals) they have no opportunity to exert it, either because their metabolism does not produce the colloidal mother substance of the yellow granules, or the amount of glomerular filtration of colloids is too low to give athrocytosis. But, as soon as the glomerular filter opens, the athrocytic properties, till then latent, awake and develop to the full.

From all these researches the following conclusions may be drawn. Athrocytosis is a characteristic property of the striated border segment of the Vertebrate nephron: it arises after the resorption of electro-negative colloids through the apical surface of its cells. This apical permeability is not equal all along the segment, but presents an increasing gradient of permeability from its proximal to its distal end. The degree of athrocytosis, or even its starting, depends on anatomical conditions, which do or do not allow the colloids to reach the apical surface of the cells of the brush border segment. So, athrocytosis can develop to the full in the open nephrons; in the closed

[1] See also Randerath, E., *Ziegler's Beitr.* vol. XCV (1935).

glomerular nephrons it is restricted to the particles which have been filtered through the glomerulus; it is absent in the aglomerular nephrons.

The athrocytic power of the brush border segment is incomprehensible when closed nephrons alone are considered. But if we remember the ontogenetic as well as the comparative anatomical data, we must consider the closed nephron as being the result of a long evolution which we may schematise in this way. Primitively, the nephron, as the nephridium, opened into the coelom through a wide aperture, which later turned into a peritoneal duct. Through this duct large quantities of coelomic fluid were forced into the nephron and conducted towards the outside: now, in this fluid are dissolved proteins and many other substances whose incessant loss would be detrimental to the organism. The brush border segment acted on them as an organ of recovery, whose function is witnessed by athrocytosis phenomena. When the nephron, having lost its communication with the body cavity, became closed, it received only highly dispersed colloids filtered through the glomerulus: since then its athrocytic function seems to have become imperceptible or even abolished. But it is only dormant; in pathological conditions it awakes and appears as developed as in the open nephrons.

Therefore the athrocytic power in the closed glomerular nephrons must be regarded as an ancestral character, transmitted through heredity, which has been kept intact, although under normal conditions it has no opportunity to reveal its existence.

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VARIATIONS IN THE CORTICAL LIPOID OF THE RABBIT SUPRARENAL WITH SEX AND AGE

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THE literature of the rabbit suprarenal contains many casual observations on cortical lipoid, but the present paper appears to be the first systematic study of the variations in the cortical lipoid of the rabbit suprarenal with sex and age.

MATERIAL AND METHODS

The suprarenals of seventy male and seventy female rabbits were studied. The rabbits were of various colours. The date of birth of each rabbit under 1 year old was known. The females under 1 year old were nulliparous; those over 1 year old had been used for breeding. The diet consisted of hay, turnips, greens, oats, bran, thirds, Uveco and water. The rabbits were killed by fracture of the neck and weighed.

Treatment of suprarenals. Both suprarenals from each rabbit were fixed in 10 per cent. formol saline for 2 days. Transverse frozen sections of each gland were (*a*) stained with Sudan IV in 70 per cent. alcohol and haemalum, (*b*) treated by the Schultz method for cholesterol. The use of Sudan IV is discussed by Kay and Whitehead (1935) and the Schultz reaction by Whitehead (1934 *a*).

Definitions. The structure designated "cortex" is morphologically equivalent to the permanent cortex of the mouse suprarenal. The term "lipoid" is used to denote "substances in normal cortical cells stained by Sudan IV". The term "lipoid density" is used to denote "the size and number of lipoid droplets in a unit area". The existence of differences in lipoid density was established by repeated comparisons under the microscope.

RESULTS

The body weight of each rabbit is shown in Table I.

Table I. *Body weights of normal rabbits*

Males							Females						
<1	14	28	56	87	168	>365	<1	14	28	56	87	168	>365
58	185	420	660	1320	2300	3120	63	270	255	550	890	1230	2290
50	145	260	600	1000	1160	2050	61	195	255	510	870	1220	2260
45	140	225	580	720	1130	1980	58	165	240	450	740	1180	2220
43	120	220	570	700	970	1950	53	140	230	440	740	1150	2140
43	120	210	520	670	970	1900	50	140	220	420	700	1060	1970
43	120	200	470	660	910	1890	43	130	220	420	700	930	1950
40	110	190	370	550	850	1800	41	100	200	400	690	850	1750
40	95	180	350	500	780	1800	40	95	175	350	640	840	1550
38	90	145	340	500	560	1680	39	85	170	300	550	790	1500
37	90	140	340	450	500	1370	35	85	170	195	520	680	1290
44	122	219	480	707	1013	1954	48	141	214	404	704	993	1892

Top row: age (days). Columns: weights (gm.) in decreasing order downwards.
Bottom row: mean weights.

General post-mortem findings

The only abnormalities found were (1) several sharply defined, spherical or oval, firm white nodules 2–4 mm. in diameter, apparently of inflammatory origin, in the liver and/or spleen, and (2) parasitic cysts in the liver and/or peritoneal cavity. The incidence of each abnormality is shown in Table II, units column.

Table II. *Types of inner lipoid band in suprarenal cortex and post-mortem findings in other organs of normal rabbits*

Males							Females						
<1	14	28	56	87	168	>365	<1	14	28	56	87	168	>365
10	10	10	10	40	30	32	10	10	10	10	10	20	10
10	10	10	10	10	10	40	10	20	20	10	10	10	10
10	10	10	10	10	10	40	10	10	10	10	10	10	10
10	10	10	10	20	10	12	10	10	10	10	10	12	11
10	10	10	10	10	30	40	10	10	10	10	10	10	10
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10	10	10	50	10	11	12	10	10	10	10	20	30	13
10	10	10	10	20	20	10	10	10	10	10	10	10	10
10	10	10	30	40	30	30	10	10	10	10	10	10	10

Top row: age (days). Tens column: 1–4 = various types of inner lipoid band, 1 = continuous, 2–4 = streaky; in 1–4 band was of same type in both glands, 5 = band of type 2 in left gland, type 1 in right. Units column: post-mortem findings, 1 = inflammatory nodules in liver and/or spleen, 2 = parasitic cysts in liver and/or peritoneal cavity, 3 = presence of both 1 and 2, 0 = absence of both. Body weight appears in corresponding position in Table I.

Table II shows that such abnormalities were first seen in males at 87 days and in females at 168 days, and that of the ten rabbits showing abnormalities seven were over 1 year old.

Involution of suprarenal cortex by fatty change

The suprarenal cortex showed involution by fatty change in two females over 1 year old, weighing 2220 and 2140 gm. An account of the phenomenon in three female rabbits over 1 year old, including these two, has been given elsewhere (Whitehead, 1935 *a*). Evidence of a structure resembling the transitory cortex of the mouse suprarenal was not found in the material under review.

General distribution of lipoid in suprarenal cortex

In every gland lipoid was present in all parts of the cortex, including a variable, usually high, proportion of cortical cells mingled with those of the medulla. In the cortex proper, lipoid was arranged in two bands; the outer band corresponds to the subcapsular rim of cells, the inner band to the rest of the cortex. In the outer band density was relatively low, in the inner band relatively high (Plate I, fig. 1).

Breadths of lipoid bands at each age

The breadth of each band was measured with a micrometer along one radius of each section; the results are shown in Table III; since the results for left and right glands agreed closely, only the means for left and right glands together are given.

Table III. *Breadths of lipoid bands in suprarenal cortex of normal rabbits*

		Age in days						
		<1	14	28	56	87	168	>365
♂	*O*	0·06	0·11	0·07	0·10	0·09	0·11	0·14
	I	0·14	0·20	0·41	0·52	0·71	1·02	1·82
	O/(*O*+*I*)	0·30	0·35	0·15	0·16	0·11	0·10	0·07
♀	*O*	0·04	0·10	0·08	0·11	0·12	0·13	0·17*
	I	0·17	0·22	0·39	0·56	0·71	0·99	1·83*
	O/(*O*+*I*)	0·19	0·31	0·17	0·16	0·14	0·12	0·09

O, *I* = breadth (mm.) of outer, inner lipoid band. The values are means for 20 glands from 10 rabbits except those marked *, which are means for 16 glands from 8 rabbits; glands from other two not measured because cortex showed involution by fatty change.

Table III shows that the breadth of the outer band increased rapidly between birth and 14 days, thereafter showing only a slight tendency to increase; the breadth of the inner band, however, increased throughout the period investigated. The values of the ratio $O/(O+I)$, where O and I denote the breadths of the outer and inner bands respectively, show that the outer band occupied about one-third of the breadth of the cortex at 14 days, but under one-tenth in rabbits over 1 year old. Corresponding results for the two sexes are closely similar.

Histological types of lipoid distribution

In the outer band density from without inwards either remained approximately constant, increased, or decreased, but as more than one of these variations often occurred in one and the same section, it was impracticable to regard them as distinct types.

In the inner band four types of lipoid distribution were observed.

Type 1. The whole band appeared as a continuous sheet of high density, which remained approximately constant either throughout the breadth of the band (Plate I, fig. 2) or in its outer part only, then decreasing slightly (Plate I, fig. 1).

Type 2. Lipoid was continuous and of high density in the outer part, streaky in the inner part (Plate I, fig. 3).

Type 3. Lipoid was continuous and of high density in the outer part, of low density in the middle part, and streaky in the inner part (Plate I, figs. 5 and 6).

Type 4. Lipoid was streaky throughout the breadth of the band (Plate I, fig. 4).

The incidence of each type is shown in Table II, tens column.

Types 2–4 are henceforward referred to collectively as "streaky types".

Variations in lipoid density

(*a*) *With age.* In the outer band density increased from birth to 14 days, and differences in density between glands from rabbits of the same age were slight; at later epochs wider variations were seen, lipoid being scanty in some glands, in others only slightly less dense than in the inner band.

Streaky types of inner band were rare up to 28 days but relatively frequent from 56 days onwards. In type 1 density increased from birth to 14 days, thereafter remaining approximately constant.

(*b*) *With sex.* Evidence of a sex difference in the appearance of the outer band was not obtained.

Individual types of inner band had the same appearance in both sexes; streaky types were commoner in males than in females.

(*c*) *With side.* The two glands from any one rabbit were closely similar in appearance; with only one exception the inner band was of the same type in both left and right glands.

Distribution of cholesterol in lipoid

After the Schultz reaction all the lipoid, including that in cortical cells mingled with those of the medulla, appeared blue-green in all the suprarenals, and therefore contained cholesterol. The appearances after Sudan IV and after the Schultz reaction differed only in colour; cholesterol density in any gland was thus the same as lipoid density, and the variations in cholesterol density were the same as those in lipoid density.

DISCUSSION

The streaky types of lipoid distribution

The percentages of males and females showing streaky types of inner lipoid band were 27·14 and 11·43 respectively; the difference, $15{\cdot}71 \pm 6{\cdot}67$, is formally though not highly significant; the results thus suggest that a streaky distribution of lipoid is more common in males than in females.

Of the total 140 rabbits, 1 showed a streaky type of lipoid distribution and visceral lesions also, 26 showed streaky types but no lesions, 9 showed lesions only, and 104 neither streaky types nor lesions. The relation of the two attributes could not be tested statistically because in one instance the number to be expected on the hypothesis of independence was too small; the above observations, however, suggest that there is no relation between streaky types of lipoid distribution and the visceral lesions.

Cortical lipoid in rabbit, guinea-pig and mouse

The results on the rabbit suprarenal may be compared with those on the suprarenal in the mouse (Whitehead, 1933, 1934 *a*) and guinea-pig (Whitehead, 1934 *b*, 1935 *b*).

The outer lipoid band in the rabbit suprarenal corresponds to the subcapsular rim of cells in the guinea-pig and mouse suprarenals, the inner lipoid band in the rabbit suprarenal to the lipoid-laden area in the guinea-pig suprarenal and the lipoid band in the mouse suprarenal.

Resemblances. In all three species (*a*) in older animals lipoid was relatively more abundant in females, (*b*) lipoid was most variable in the inner part of the cortex, (*c*) all the lipoid contained cholesterol, and (*d*) the two glands from any one animal were closely similar in appearance.

Differences. (*a*) In the rabbit, but not in the guinea-pig or mouse, lipoid was always present in all parts of the cortex, (*b*) in the rabbit, lipoid in the subcapsular rim of cells was more abundant than in the guinea-pig or mouse, (*c*) in the rabbit and guinea-pig, but not in the mouse, cortical cells, many of which contained lipoid, were observed in the medulla, and (*d*) in the rabbit and mouse, lipoid density was more variable than in the guinea-pig.

SUMMARY

1. The distribution of cortical lipoid in the suprarenals of 120 normal rabbits of known ages from birth to 168 days, and of 20 normal rabbits over 1 year old, has been studied.

2. In every gland lipoid was present in all parts of the cortex, including cortical cells mingled with those of the medulla.

3. Lipoid usually appeared as a continuous sheet, but in some glands it was streaky.

4. Streaky types of lipoid distribution were rare up to 28 days but relatively frequent thereafter; they were commoner in males than in females.

5. There was no evidence of a relation between streaky types of lipoid distribution and spontaneous disease.

6. In every gland all the lipoid contained cholesterol, cholesterol density was the same as lipoid density, and the variations in cholesterol density were the same as those in lipoid density.

7. The two glands from any one rabbit were closely similar in appearance.

8. The results are compared with those obtained on the guinea-pig and mouse.

I am indebted to Mr F. W. Priestley for advice on statistical problems and to Prof. S. L. Baker for criticism. The cost of the work was borne by the University committee on grants in aid of medical research.

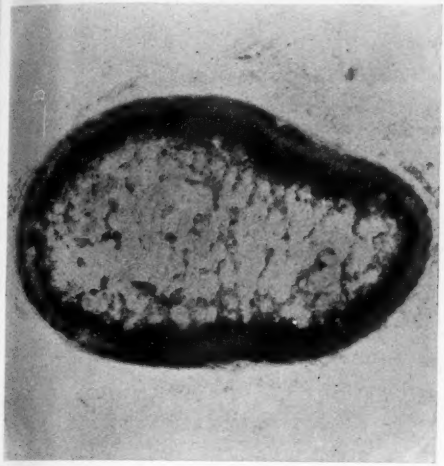

Fig. 1

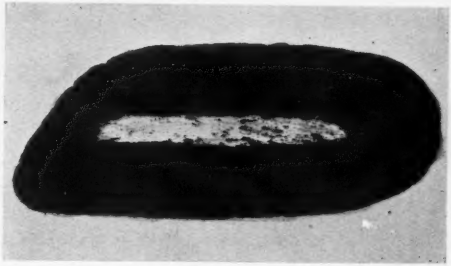

Fig. 2

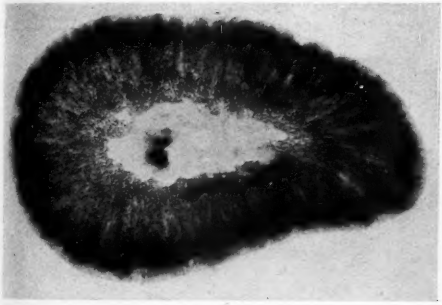

Fig. 3

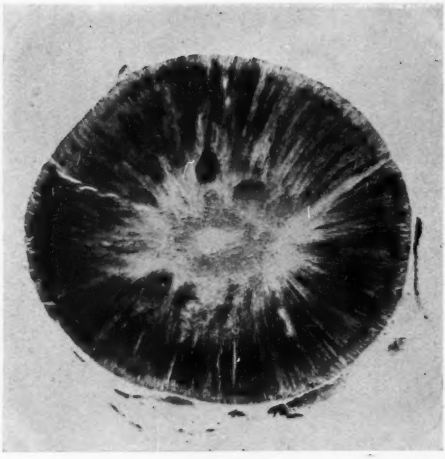

Fig. 4

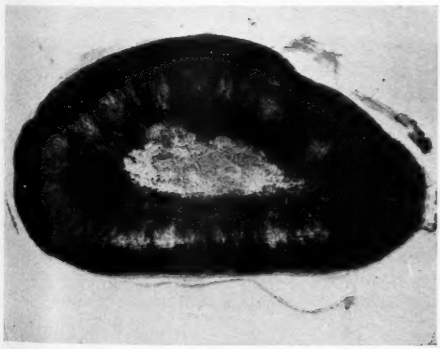

Fig. 5

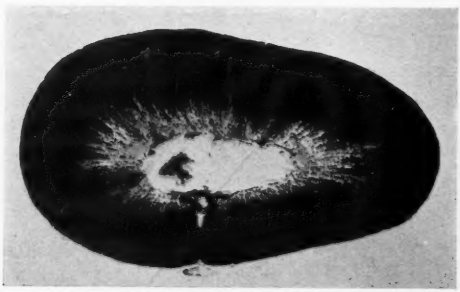

Fig. 6

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EXPLANATION OF PLATE I

Figs. 1–6 show transverse frozen sections, stained with Sudan IV and haemalum, of suprarenals from normal rabbits. Lipoid appears black or dark grey, medulla light grey or colourless; dark areas in the medulla represent groups of lipoid-laden cortical cells. For each figure the breadth of the outer lipoid band is given; this is its breadth in the figure, not its absolute breadth. (Photographs by H. C. Taylor.)

Fig. 1. Outer and inner lipoid bands are each approximately 3 mm. in breadth. Inner band of type 1; lipoid density is greater in the inner band than in the outer band; in the inner band, density decreases slightly towards the medulla. Many lipoid-laden cortical cells are present in the medulla. Left gland from a female under 1 day old. ×35.

Fig. 2. Outer band occupies outermost 1 mm., or less. Inner band of type 1; density is uniformly high throughout its breadth. Right gland from a female 87 days old. ×16.

Fig. 3. The whole cortex is occupied by lipoid, which appears dark grey except in the outer quarter of the inner band, where it is black. Outer band occupies outermost 2 mm. Inner band of type 2; lipoid in its outer quarter is of high density; in its inner three-quarters, streaky. Left gland from a male 87 days old. ×19.

Fig. 4. Lipoid is present in all parts of the cortex, but near the medulla is too scanty to be clearly visible at this magnification. Outer band occupies outermost 2 mm. Inner band of type 4; lipoid is streaky throughout its breadth. Left gland from a male over 1 year old. ×10.

Fig. 5. Outer band occupies outermost 1 mm., or less. Inner band of type 3; lipoid in outer third is of high density; in middle third, of low density; in inner third, streaky. Left gland from a male 87 days old. ×18.

Fig. 6. Outer band, where visible, is less than 0·5 mm. in breadth; the black strip 2 mm. broad at the periphery of the figure is part of the inner band. Inner band of type 3; it resembles that in fig. 5, but the inner streaky part is relatively broader. Left gland from a male over 1 year old. ×8.

STUDIES ON THE CARDIAC ANATOMY OF THE ELEPHANT

I. THE CORONARY BLOOD VESSELS

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The heart of the elephant was the first part of the anatomy of that animal to be studied, Aelian, the authority of ancient Rome, having stated that this creature had two hearts, being misled by the bifurcation at the ventricular apex. Galen mentioned an *os cordis*, a structure which has been denied by recent observers. John Hunter was the first to note two precaval veins. After this few observations were made on elephantine anatomy until the appearance of Camper's posthumous writings (1803). Since then many elephants have been dissected in Europe, but all of them have been specimens that had died in captivity. Few of them were dissected thoroughly, since none of them was embalmed. Most of the dissections were rough and ready post-mortem examinations, and in only a few instances were large parts or organs preserved separately for further detailed study. The heart received scant attention, having been mentioned only by Stukeley (1723) and Mayer (1857). These two observers confirmed the presence of a bifurcated apex, whilst Mayer studied the valves and the great vessels, and noted the ductus arteriosus. He did not mention the species of elephant described; whilst many of his statements have not been confirmed by more recent observers. The first detailed account of the Indian elephant's heart was the classic one of Vulpian and Philipeaux (1856). This was based on an isolated preserved heart; it was not complete, and the description of the coronary arteries was but a bare outline, though the veins were dealt with in more detail. Their specimen did not show the bifurcated apex.

Morrison Watson published a series of papers on elephantine anatomy between 1871 and 1878. The part containing the description of the heart appeared in 1872. He discussed the main blood vessels and the valves. His account of the anterior veins was vague.

The first modern account of the anatomy of an African elephant was that of Forbes (1879). His mention of the heart was brief. He did not note a bifid apex. The fossa ovalis was very deep. The ductus arteriosus admitted a quill pen for short distance only. The coronary vessels were not mentioned. A. von Mojsisovics (1879) also studied the African elephant, but he made no mention of the heart. In the same year appeared the classic memoir on the Indian elephant by Miall and Greenwood (1879). In this work the heart was re-

described and some new details added. The coronary vessels received only scant attention, two coronary arteries being mentioned, whilst the great cardiac vein was stated to open into the left precaval vein. Statements in the treatises of Owen (1868), Huxley (1871) and Leche (1902) are based on the work of the previously mentioned authors. The only other account that need concern us is that on the anatomy of a foetal African elephant by Nellie B. Eales (1929). This contained some important new observations as well as a good historical review of the earlier work.

The opportunity of studying a complete heart removed from a shot wild adult male Ceylonese elephant (*Elephas maximus zeylanicus*) was kindly afforded me by Mr A. H. Malpas, Director of the Colombo Museum. Dissection revealed a number of interesting points, especially in connection with the coronary vessels. Comparison with the previous literature mentioned above showed that there was much to be added to the known facts; hence the more detailed account which follows.

GENERAL APPEARANCE OF THE HEART

The specimen was received hardened in formalin, in rather bad shape, having been fixed in a kerosene tin. The fixation was, however, complete, so that it was a relatively easy matter to orientate the organ. The surface was almost everywhere covered by a thick layer of serous pericardium, under which lay a very thick mass of subepicardial fat. This is interesting in so far as fat has been mentioned by previous dissectors as being absent from the subcutaneous and other tissues of captive elephants. The only account which mentions fat on the heart is that of Vulpian and Philipeaux, who found fat at the base and on the front of the ventricles. Miall and Greenwood definitely state that no fat was present in their specimen. In the present specimen the fat varied in thickness and appearance on different parts of the surface of the organ. It was thinner over the convex surfaces of the ventricles and on the atria, and thick over the coronary and interventricular sulci, all of which it completely masked. The thickness here may be judged by the depth of the sulci which were as follows:

Coronary sulcus in front	45 mm.
Coronary sulcus, behind	25 ,,
Ventral interventricular sulcus	50 ,,
Dorsal interventricular sulcus	25 ,,
Depth at posterior crux	90 ,,

Fat was thinner towards the apex of the ventricles, but was sufficiently developed to mask the bifurcation. When the fat was removed the bifid apex was evident, but not so marked as would be expected from the accounts of previous observers.

The more superficial part of the subepicardial fat was firm in consistence and pale yellow in colour. The deeper stratum was darker and of softer con-

sistence. The fat was separated from the muscular tissue of the heart by a definite stratum of aerolar connective tissue. The coronary vessels were completely enveloped in the fat, and their more superficial branches ramified within it. This applied equally to atria and ventricles. The fat thinned out and ceased at the origins of the great vessels, especially the great veins. The commencement of the pulmonary and systemic aortae was deeply embedded in fat.

Measurements

The following measurements were taken on the heart after removal of the subepicardial fat and areolar tissue:

Weight: 10·8 kg. (24 lb.)

Size of great vessels:	Diameter mm.	Thickness of wall mm.
Systemic aorta	97	11
Pulmonary aorta	55	6
Right precaval vein	58	1
Left precaval vein	48	1
Postcaval vein	101	2
Pulmonary vein (i)	60	—
,, (ii)	60	—
,, (iii)	38	—
,, (iv)	35	—
,, (v)	18	—
Right coronary artery	16·5	—
Left coronary artery	17	—

THE ARTERIAL SUPPLY OF THE HEART

The heart is supplied with blood from three arteries, all of which spring from the root of the systemic aorta. These are the right and left coronary arteries and a third artery solely destined for the supply of the two atria. This third vessel seems to have escaped the notice of previous observers, although it is a large artery and easily traced out to its ultimate distribution. It will be described here as the atrial artery.

The root of the systemic aorta lies deeply behind that of the pulmonary trunk. It is dilated to form three sinuses as in other mammalian hearts. These are situated two ventrally and one dorsally, corresponding in position to the cusps of the aortic valve. Vulpian and Philipeaux state that the origins of the two coronaries are situated at such a level that the valve cusps can successfully occlude half the coronary orifice, and they infer from this that the coronaries are filled not during ventricular systole, but in the period when the ventricles are in diastole and the atria in systole. This view, they affirm, is further supported by the fact that both vessels take a recurrent course. In my specimen the semilunar cusps barely reach the inferior limits of the coronary orifices, but I have no doubt that in the fresh state, before the contraction due to the preservative had occurred, the conditions were as stated by the above authors. Only the left coronary artery can be said to have a recurrent course, however, since the right vessel runs for a short distance parallel with the aorta before turning off towards the right margin of the heart.

(1) *Left coronary artery*

This vessel arises from the left ventral aortic sinus. Its orifice is funnel-shaped and admits two fingers with ease for a distance of about 2 cm. The vessel then rapidly narrows to a diameter of 15 mm. The first portion of the artery before the narrowing is markedly compressed against the aortic wall, both in a dorso-ventral and a cephalo-caudal direction. Beyond this funnel-shaped commencement the vessel assumes the normal cylindrical form. The

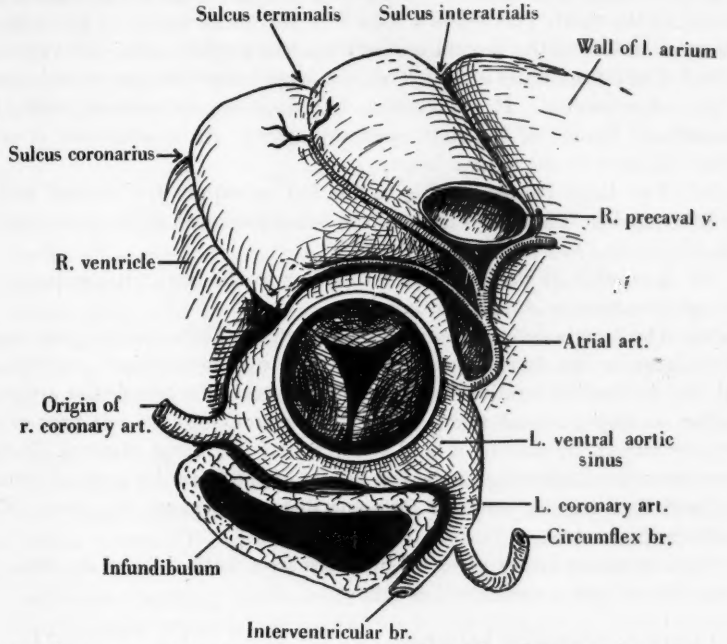

Fig. 1. Diagram of the root of the aorta with the origins of the coronary arteries and neighbouring structures.

direction of the main stem is recurrent with respect to the direction of its parent vessel, the artery proceeding caudally. It is short, having a total length of only 50 mm., before dividing into two terminal branches. The relations of the left coronary artery are as follows: it is completely hidden ventrally by the trunk of the pulmonary artery; behind it in its upper funnel-shaped half is the aortic wall, and in its lower half the ventricular muscle. At its origin it is separated by a space of 50 mm. from the origin of the atrial artery.

Branches. The only branches are the two terminal branches, namely, the interventricular and the circumflex.

The *interventricular branch* continues the direction of the main stem

caudally into the ventral interventricular furrow, passing somewhat to the right following the lower margin of the left anterior sinus of the pulmonary trunk and then along the left margin of the infundibulum into the interventricular sulcus. Along this it courses towards the apex, where it ends. It is a little over 160 mm. long and gives the following branches:

(i) A vessel passing to the right on the ventral aspect of the left ventricular wall behind the infundibulum. It ends in the ventricular muscle caudal to the root of the aorta.

(ii) A similar, but larger, vessel, arising 25 mm. below the preceding also passing to the right. This sends a branch to the dorsal aspect of the infundibulum, as well as to the ventral wall of the aortic portion of the left ventricle.

(iii) The infundibular artery. This is a large branch to the ventral aspect of the infundibulum. It approaches, but does not anastomose with, the infundibular branch of the right coronary artery. An anastomosis is usual in this situation in the human heart.

(iv) Two large vessels passing to the left to supply the ventral wall of the left ventricle. These pass deep to the commencement of the great cardiac vein.

(v) A number of smaller branches similar to the last, but passing on to the right ventricle to supply its muscle.

(vi) The terminal branches in the apical fork. These do not pass round the apex on to the dorsal aspect, nor is there any precapillary anastomosis with the descending terminal twigs from the dorsal interventricular artery.

The *circumflex branch* takes a transverse course into the left part of the coronary sulcus, by making a bold sweep round the margo obtusus. It ends after a course of 160 mm. on the left aspect of the heart by making a sharp kink and then passing caudally for some distance towards the apex, i.e. as a *left marginal artery.*

The circumflex artery gives off branches from both sides of its stem, i.e. a cephalic set and a caudal set, as follows:

(*a*) Caudal or descending branches:

(i) A small branch given off just past its origin. This dives almost immediately into the thick muscular wall of the left ventricle just below the atrio-ventricular fibrous ring.

(ii) Another similar vessel about 25 mm. farther along, near the margo obtusus.

(iii) Three large branches lying on the surface of the left ventricle about 35 mm. apart. The first one soon bifurcates but the other two remain single. The third one is the termination of the main artery after its kink at the dorsal margin of the left aspect of the left ventricle.

All these large descending branches are crossed superficially by the great cardiac vein as it travels towards the dorsal surface. The third vessel gives off a few collaterals to neighbouring parts of the musculature, but none

anastomoses, even slightly, with any twig derived from the right coronary artery.

(*b*) Cephalic or ascending branches:

(i) Opposite the margo obtusus a long ascending twig passes to the ventral margin of the left auricle, after giving some small twigs to the ventricular muscle just caudal to the atrio-ventricular fibrous ring.

(ii) A small vessel arising near the terminal kink. This could not be traced on to the atrial wall, but appeared to end in the ventricular muscle below the fibrous ring.

(iii) Ascending twigs from the first collaterals of the third descending artery (left marginal). The same remarks apply to these as to the preceding, no atrial branches being evident.

(2) *The atrial artery*

This important artery is practically the sole supply to the atria and to the roots of the great veins. It is a large vessel and has a complicated course. It arises from the left ventral aortic sinus dorsal to the origin of the left coronary artery, and also at a somewhat lower level in reference to the free edge of the semilunar cusp. It is quite separate from the left coronary, though it is conceivable that in some hearts it may arise as a branch of the latter, or at any rate from a common orifice therewith. At its origin from the aorta the atrial artery has a diameter of 8 mm. Its length to its main division is 115 mm.

The atrial artery is flattened at its origin, being compressed between the systemic aorta and the left auricle wall. It courses sharply dorsalwards in the groove between the aortic wall and the medial aspect of the left auricle (atrial appendix), and, following the curvature of the aortic wall till it attains the mid-dorsal position, it thereafter ascends over the margin of the atrium towards the right precaval vein near which it ends by breaking up into a series of branches which radiate fanwise. Aside from these terminal ramifications the atrial artery has a single collateral branch. This is given off from the main stem just prior to its mounting the atrial wall from the atrio-aortic sulcus. This branch continues the direction of the main stem along the atrio-aortic sulcus, only it is now in relation to the right atrial wall instead of the left. It ends in the musculature of the right atrial wall.

Terminal branches. These are four in number, all more or less of the same size, and but little inferior in that respect to the parent vessel. The branches are described below in order from right to left.

(i) The first passes between the crest-like cephalic border of the right auricle and the trunk of the right precaval vein. It gains the sulcus terminalis, along which it runs and supplies terminal twigs to the right and left. It has no anastomoses with other vessels.

(ii) This is a continuation of the parent vessel and after a short distance (10 mm.) it divides into two:

(α) A twig which ascends on the wall of the right precaval vein.

(β) A larger vessel which passes into the interatrial sulcus on the dorsum of the heart. It passes caudally in this sulcus, lying deeply between the walls of the two atria, being hidden on the left by the anterior left pulmonary vein. Half-way along the sulcus it turns to the left on to the wall of the left atrium between the posterior sets of pulmonary veins and the left precaval vein running thence transversely to the extreme left of the left atrium, where it appears to end in the muscular tissue of the wall of the left pulmonary veins.

(iii) A branch to the wall of the right precaval vein.

(iv) A branch to the roof of the left atrium. This has a transverse course and ends in the atrial muscle. A similar vessel running parallel to it is derived from (β) above before this latter vessel enters the interatrial sulcus.

(3) *Right coronary artery*

The right coronary artery springs from the right ventral aortic sinus at a slightly higher level than the left. Its origin is not funnel-shaped like the left, but has the usual cylindrical form, with a diameter of 18 mm. Its length from its origin to the crux is 40 cm. For about 25 mm. the vessel follows the course of the aortic trunk, against which it lies, but then it gradually inclines dorsally. In the first part of its course it has the aortic wall on its left, the dorsal aspect of the infundibulum ventrally, the convex wall of the right ventricle on its right, and the ventral aspect of the right atrial wall dorsally. This last is separated by a considerable space, filled in the recent state with fat. After inclining dorsally the vessel mounts the crest-like margin of the right ventricular wall and courses along it till it reaches the atrio-ventricular furrow. It follows this as far as the crux dorsally, where it divides into two terminal branches, both of which pass deep to the termination of the middle cardiac vein. The right coronary artery gives off both collateral and terminal branches.

(i) *Collateral branches.*

These may be arranged in four groups as follows:

(*a*) Two small branches coming off immediately after the origin of the main vessel. They dive at once into the muscular substance on the dorsum of the infundibulum.

(*b*) A ventral infundibular artery. This corresponds to the infundibular branch in Man. It courses round on to the sternal aspect of the infundibulum and ramifies thereon without forming any communication with the corresponding branch of the left coronary. Before attaining the sternal surface it furnishes a twig to the dorsum parallel with those mentioned under (*a*) above.

(*c*) One or two small vessels to the prominent cephalic border of the right ventricular wall.

(*d*) Three medium-sized branches passing apically on the right aspect of the right ventricle. None of them reaches the apex. A fourth similar vessel is given off just prior to the crux.

(ii) *Terminal branches.*

These are two in number, transverse and interventricular.

(*a*) Transverse terminal branch. This continues the course of the main vessel into the left half of the coronary sulcus on the dorsal aspect of the heart. It passes beneath the large veins ascending from the right ventricle to reach the right atrium and then comes to lie below the terminal part of the coronary venous sinus. It accompanies a tributary of the latter which is coming from the left ventricular wall, and then passes towards the apex. Some of its final

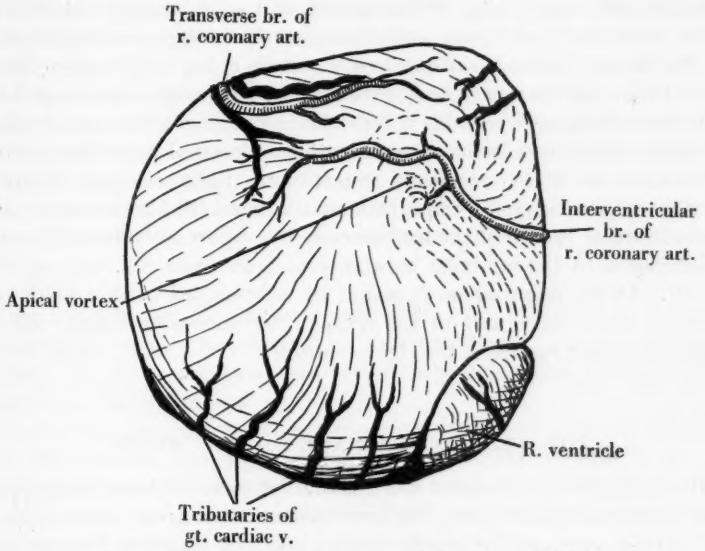

Fig. 2. Arrangement of blood vessels at the apex of the heart.

ramifications approach very closely to the apical vortex, but the main vessel turns somewhat aside and terminates in the left ventricular muscle 50 mm. to the left of the termination of the interventricular branch.

The transverse terminal branch of the right coronary artery is distributed mainly to the left ventricular wall, but there is also an atrial supply. The arrangements are as follows: Three or four small ascending twigs end in the upper part of the left ventricular wall, just below the atrio-ventricular fibrous ring. The first is larger than the others and comes off in the deep recess of the crux, from which it dives into the thick ventricular muscle. One only

could be traced beyond the ring on to the atrial wall for a very short distance. A large atrial branch is given off where the vessel meets the coronary sinus. This branch passes deep to the sinus, ascending obliquely into the sulcus between the left atrium and its auricle, and deeply hidden by the left precaval vein. Continuing its ascent on the atrial wall, it attains the roof of the latter and then bends to the right. It fails to reach and anastomose with the atrial artery from the aorta. Before crossing the atrio-ventricular junction this vessel gives a branch to the left ventricle.

Of descending branches there are two large and several small ones all ending in the left ventricular wall.

(*b*) The interventricular branch of the right coronary artery is the larger of the two terminal branches. For about 25 mm. it passes obliquely caudal to the transverse branch and then divides into two. Of these two vessels one is smaller and, after giving off one superficial branch, it dives into the substance of the left ventricular wall. The other is a large vessel which passes into the dorsal interventricular sulcus, in which it lies very deeply. After a course therein of 140 mm. it leaves the sulcus by making a right-angled bend to the left ventricle. Upon this it runs obliquely towards the apex, ending in the apical vortex in a series of ramifications separated some distance from the termination of the transverse branch of the right coronary. By taking the right-angled bend it escapes communication with the terminus of the interventricular branch of the left coronary by a space of 60 mm. This artery is distributed to the muscular substance of both ventricles, but mainly to the left. At the point where it makes its right-angled bend a small vessel continues for a short way in the dorsal interventricular sulcus, but soon escapes on to the surface of the right ventricle.

THE VENOUS DRAINAGE OF THE HEART

In my specimen the venous blood is drained from the heart by three large veins and some smaller ones. The large veins are the great cardiac vein, the right cardiac vein and the middle cardiac vein. Of these the first opens into the termination of the left precaval vein. The other two open directly into the lower medial part of the right atrium. This arrangement differs somewhat from the account given by Vulpian and Philipeaux, who state that the great vein opens into the atrium, whilst a vein which they term the left transverse coronary vein opens into the left precaval vein. Miall and Greenwood only mention the great vein, which they state opens into the left precaval, as in my specimen. The same condition is stated to occur in the African elephant by Eales. In this animal one other coronary vein is mentioned. This is called the small or right coronary and is due to the union of two factors, dorsal and ventral, which open close together directly into the right atrium. They evidently correspond to the veins I have named right and middle cardiac.

(1) *Great cardiac vein*

This is the largest vein and drains all the heart wall except the right and dorsal part of the right ventricle. It is formed at the apical cleft alongside the termination of the interventricular branch of the left coronary artery. In total length it measures 530 mm. and at its termination it has a diameter of 25 mm.

Its course may be divided into three parts, (i) interventricular, (ii) horizontal, and (iii) vertical. The first part accompanies the interventricular branch of the left coronary artery in the ventral interventricular sulcus. It lies superficial and nearer to the apex than the artery, and does not proceed as far as the coronary sulcus before turning to the left on its second or horizontal part.

The second or horizontal part is formed by the vein taking a bend to the left on to the surface of the left ventricle. This bend corresponds to the site where the first large descending vessel arises from the left coronary artery. From this point the great cardiac vein engirdles the left ventricle 60 mm. caudal to the line of the transverse branch of the left coronary artery.

The third part commences on the dorsal aspect of the heart by the vein bending once more into an almost vertical position and passing thence towards the coronary sulcus, which it crosses, to terminate by opening into the left caudal angle of the left precaval vein. In the third part of its course the vein is accompanied by the terminal transverse branch of the right coronary artery.

Tributaries.

Into the first part of the great cardiac vein open a number of tributaries corresponding to the branches of the interventricular division of the left coronary artery. In addition there is one large descending tributary in the region of the margo obtusus.

The second part receives seven tributaries ascending from the left aspect of the left ventricle, and one large descending tributary from the same.

The third part receives a large ascending tributary which arises near the apex and passes towards the coronary sulcus along the border separating the left from the dorsal aspect of the left ventricle.

There is no collector from the dorsal interventricular sulcus opening into the great cardiac vein as in Eales' specimen of the African species.

Semilunar folds, sometimes single and sometimes double, occur at the mouths of practically all the tributaries of the great coronary vein, even to some of the smaller ones. Some of these folds are described in detail by Vulpian and Philipeaux.

(2) *Middle cardiac vein*

This is a much smaller vein, but like the preceding is more of a sinus than an ordinary vein. It takes origin near the apex in company with the terminus of the dorsal interventricular division of the right coronary artery. It is

200 mm. long from apex to its opening into the right atrium; and 25 mm. in circumference at its termination.

Its course is an oblique one in the dorsal interventricular sulcus in company with the dorsal interventricular artery, to which it lies superficial. It crosses the two terminal branches of the right coronary artery 25 mm. below their origin. It approaches the coronary sulcus and crosses it to open into the caudal end of the right atrium on its left-hand side, 50 mm. to the right of the opening of the great cardiac vein. In the African elephant, according to Eales, this vessel opens into the great cardiac vein at its termination, as in Man.

Tributaries.

This vein receives small tributaries from the dorsal wall of both ventricles in its early course, followed by some larger ones from the back of the left ventricle. Two still larger vessels from the left ventricle are received just before its final opening into the atrium.

(3) *Right cardiac vein*

This vessel is about the same size as the last, as far as length is concerned (175 mm.), but is less in calibre, being only 12 mm. in diameter at its termination.

It commences on the dorsum of the muscular portion of the infundibulum, which is easily separable from the front of the aorta. Its origin is closely related to the origin of the right coronary artery.

Its course follows that of the main stem of the right coronary artery as far as the most lateral point on the lateral wall of the right atrium. Here it ends by opening at once into the atrium.

It drains both surfaces of the infundibulum by tributaries which correspond to the infundibular branches of the right coronary artery. In addition it receives a number of collectors from the right aspect of the right ventricular wall.

(4) *Small cardiac veins*

Only one vein falling into this category was discovered. This was a small vessel of 50 mm. length situated on the dorsal aspect of the right atrium and ascending thereon from the region of the coronary sulcus to open into the auricle (appendix) just lateral to the sulcus terminalis.

SUMMARY AND CONCLUSIONS

1. The blood supply of the heart of a wild Ceylonese elephant is described in detail.

2. The arterial supply is from three arteries all arising from the systemic aorta; right and left coronaries and an atrial artery.

3. The right and left coronaries are distributed mainly to the ventricles,

the right supplying the greater part of the right ventricular muscle and the left ventricle at the back as far left as the left margin, whilst the left coronary supplies the rest of the left ventricle and a strip of the right near the ventral interventricular furrow.

4. The atria and their auricles are supplied almost exclusively by the atrial artery, but a few small twigs and one larger vessel pass from the transverse terminal branch of the right coronary on to the left atrium, whilst the left auricle also receives some minute vessels from the transverse branch of the left coronary.

5. No anastomoses were found among any of the arteries.

6. The venous drainage of the heart is by three large vessels and some smaller ones. The largest is the great cardiac vein, which drains the left ventricle and a strip of the right in the neighbourhood of the ventral interventricular furrow. Next is the middle cardiac vein which drains the dorsal part of the left ventricle and part of the right. Thirdly the right cardiac vein drains the remainder of the right ventricle.

ACKNOWLEDGMENTS

In conclusion I have to express my warmest thanks to Mr A. H. Malpas, Director of the Colombo Museum, for the gift of the heart upon which my observations are based. I have also to thank him for much trouble in obtaining literature not available in Ceylon. I also wish to express my indebtedness to Dr A. J. E. Cave, of the Royal College of Surgeons, for assistance with other literature.

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EXPLANATION OF PLATES I–III

Plate I

View of the heart from the ventral aspect and slightly from the left, to show the origin, branches and distribution of the left coronary artery, and the greater part of the course of the great cardiac vein.

Plate II

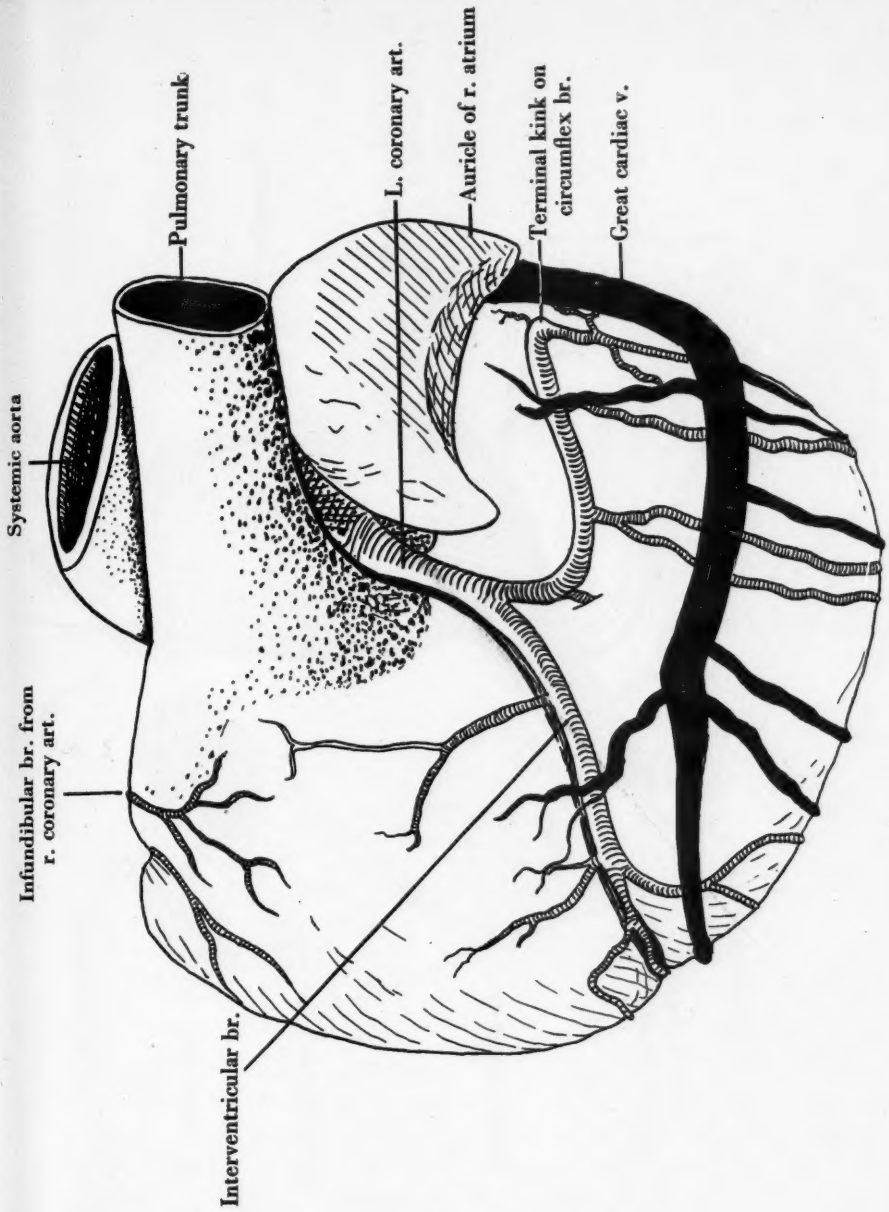
View of the heart from the base and somewhat to the right to show the origin of the right coronary artery and the course and termination of the right cardiac vein.

Plate III

View of the heart from the dorsal aspect and slightly to the right to show the further course and branches of the right coronary artery, the termination of the atrial artery and the termination of the great cardiac and middle cardiac veins.

ADDENDUM

I am indebted to Dr Cave for an examination of an Indian elephant's heart, preserved in the stores of the Royal College of Surgeons' Museum. In this specimen but two vessels, the right and left coronary arteries, arise from the aorta; these have in general a course and distribution in agreement with that outlined in this paper. No special atrial artery arises either from aorta or left coronary, but a large atrial branch is provided by the right coronary artery. This atrial vessel courses dorsally between the root of the aortic trunk medially and the right atrium laterally and expends itself almost entirely in the supply of the latter.



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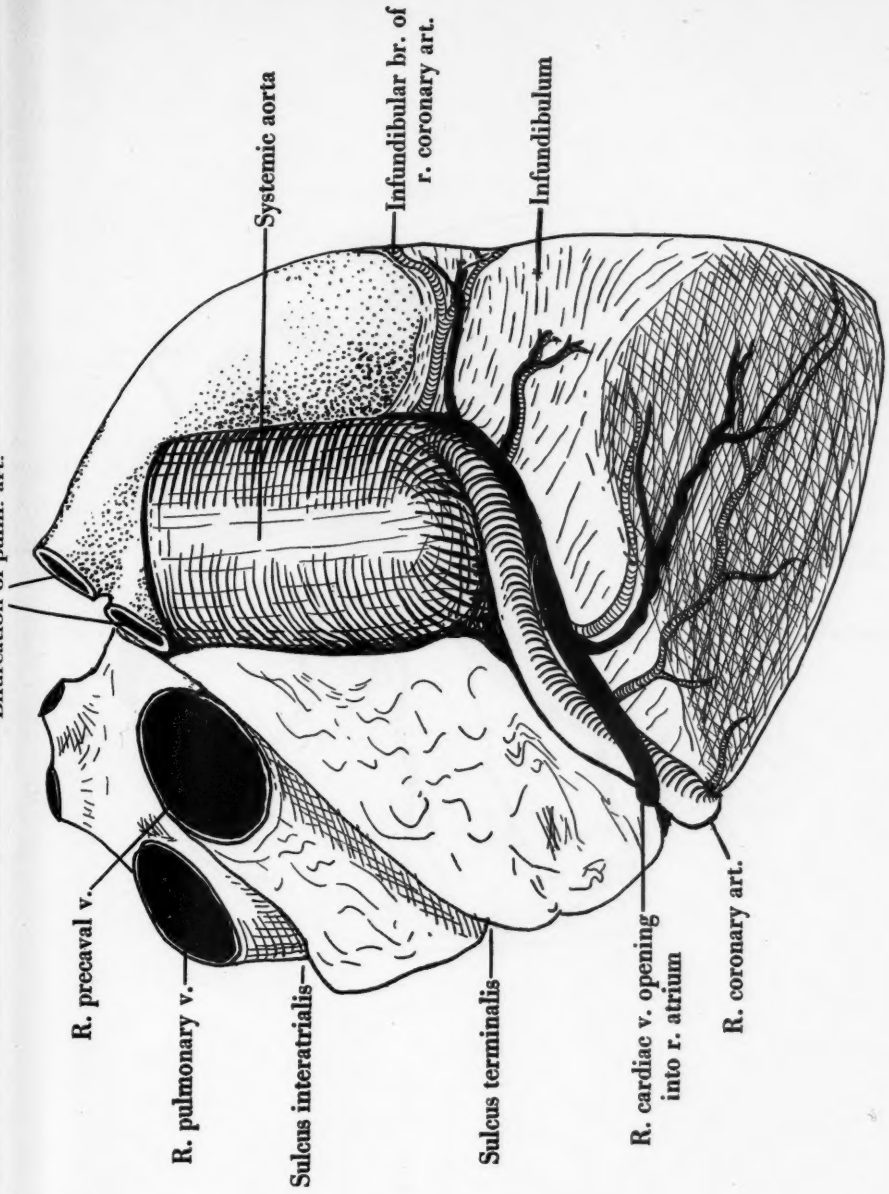

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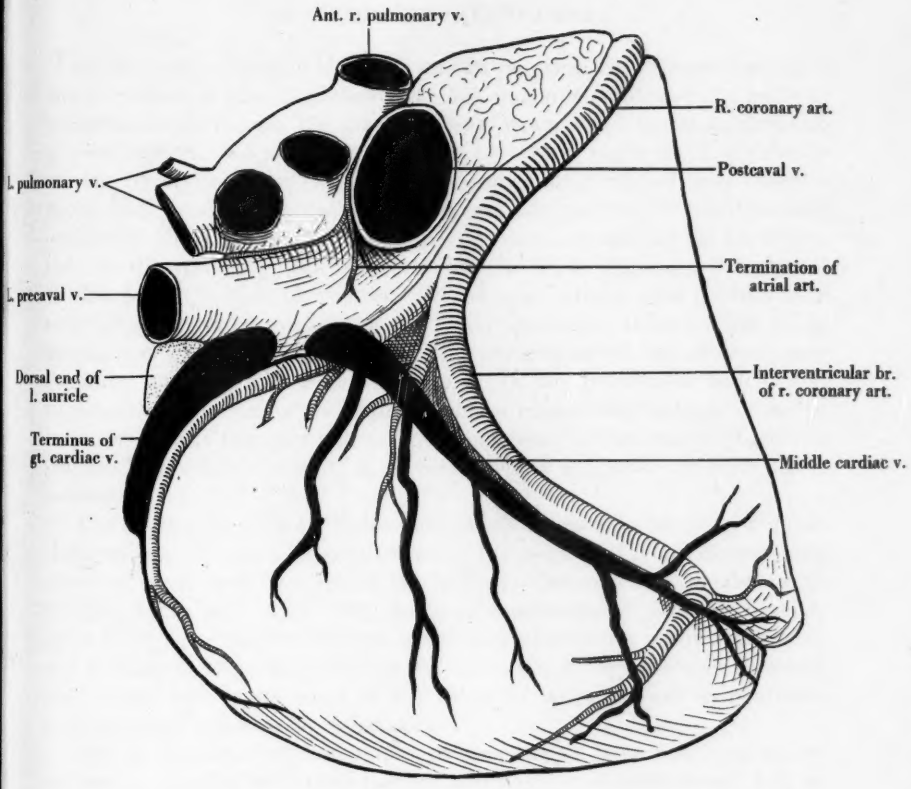
Ant. r. pulmonary v.
R. coronary art.
Postcaval v.
Termination of atrial art.
Interventricular br. of r. coronary art.
Middle cardiac v.
Dorsal end of l. auricle
Terminus of gt. cardiac v.

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SKULLS FROM THE PURARI PLATEAU, NEW GUINEA

By FREDERIC WOOD-JONES

The discovery of gold in the great central ranges of New Guinea has led to the revelation of what is probably the last romantic addition to be made to ethnological knowledge. The great central plateau, which lies at an elevation of over 5000 ft., has now been traversed by several explorers. The pioneers of this exploration—the Leahy brothers—have not published their observations, made upon several prospecting journeys, nor have their several thousand admirable photographs been reproduced by them, though several have been used to illustrate the work of others. Mr E. W. P. Chinnery, Director of Native Affairs in New Guinea, has already made ethnologists familiar with some of the peculiar customs of the newly discovered tribes (1). Mr K. L. Spinks has given good accounts of the topography of the plateau—now generally known as the Purari Plateau (2). Of the remarkable and diverse cultural developments of the plateau tribes a considerable amount of knowledge has already been gleaned; and the unpublished official reports of District Officers Taylor and (later) A. A. Roberts contain a mass of accurate observations.

The latest reports by Mr Hides, though having gained a far wider popular currency, are of minor importance when the work of Taylor, Roberts and others is considered. By Taylor the tribes are estimated to number some 200,000 people, and very possibly this is an underestimate. As white contact, in the form of Government Patrols, miners and missionaries, is now established and is likely to prove disastrous to the natives, it is very much to be hoped that a thorough examination of the tribes by a trained and sympathetic anthropologist will soon be undertaken.

Several hundreds of photographs of these people have now been taken, and we are familiar with their features and physical characteristics; but, so far as I know, no crania have as yet been examined or described. I am fortunate that, through the kind offices of Captain McLaren, I have had the opportunity of examining four skulls, all in a good state of preservation and two of them possessing mandibles. These skulls are all of adult males and all were said to be the victims of the almost constant intertribal warfare. All are recent and one had a considerable amount of muscle and tendon still adhering to it. Thanks also to the same donor, I have had the opportunity of comparing these Purari skulls with those of other New Guinea natives from Wewak and Bulolo, districts that are far removed from the Purari Plateau. A summary of the main features of these Purari skulls, which obviously con-

stitute a homogeneous series, is as follows. The form of the calvarium in norma verticalis is ovoid, with a tendency to be somewhat angulated. There is no obvious cranial asymmetry in any skull, and the average cranial index is 75·6. In norma facialis there is some tendency towards keeling of the vault. The brow ridges are prominent and confluent in the mid-line. The orbits are

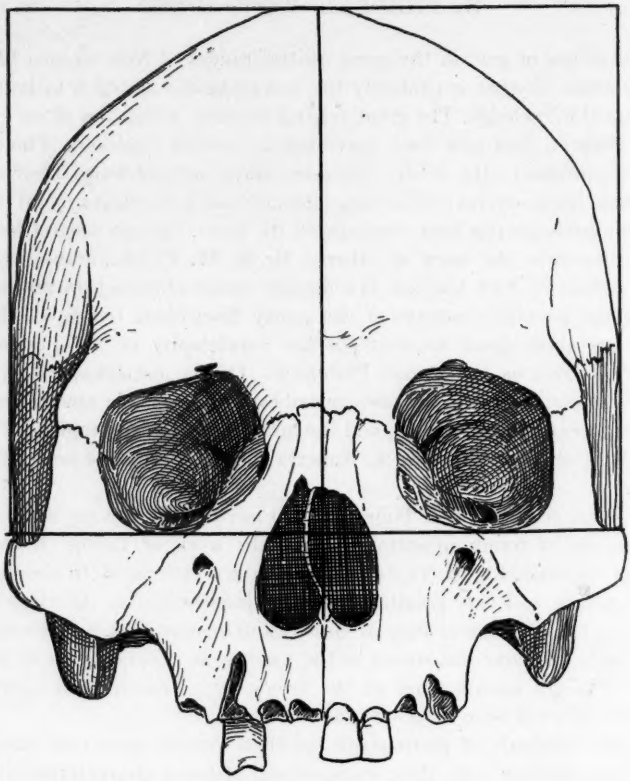

Fig. 1. Norma facialis. Benabena. Purari. ♂ A.

square and oblique. The root of the nose is depressed, the bridge elevated. The narial aperture is wide, the average nasal index being 57·3, and the margins are guttered. The guttering of the narial margins is associated with a well-marked subnasal prognathism, the average alveolar index being 107; but the chin is fairly well developed and tends to be rounded. The occiput, in norma lateralis, is produced and not flattened, and the external occipital protuberance is extremely well developed. The forehead, above the brow ridges, is retreating. The teeth are large and well formed, the average length of the molar series is 28·5, the first molar being the largest. The "anterior

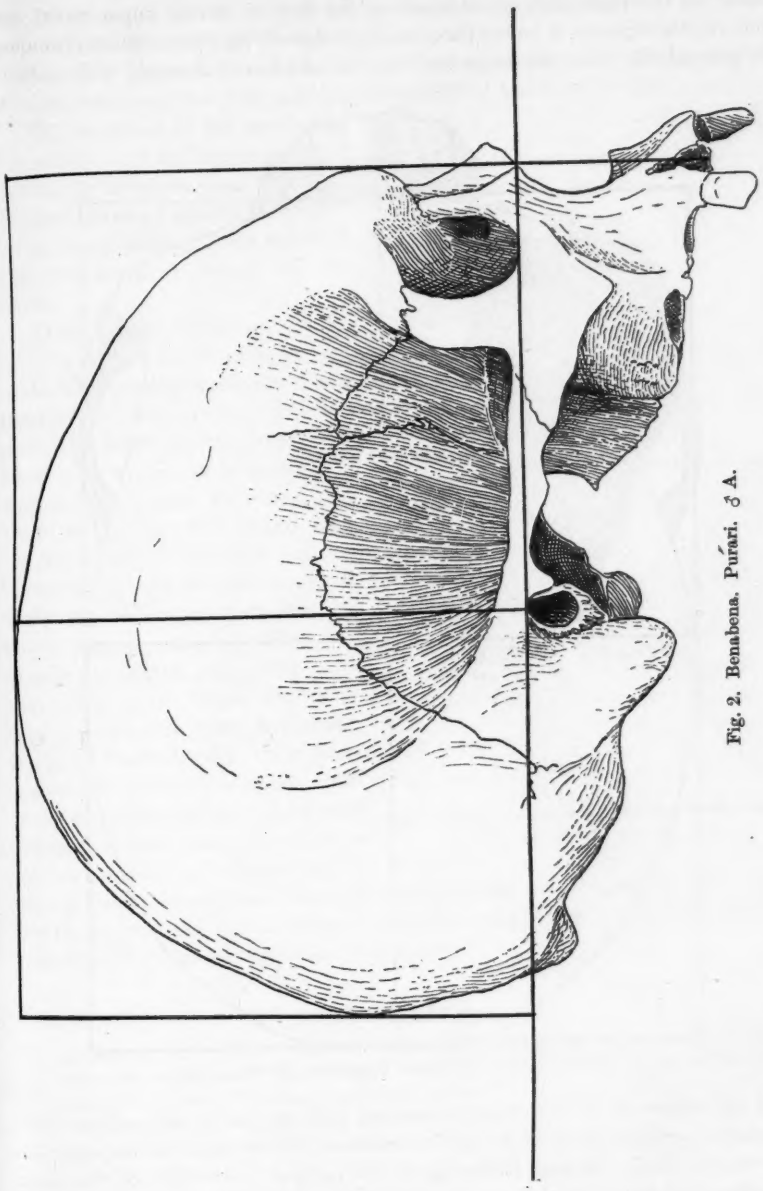

Fig. 2. Benabena. Purari. ♂ A.

root" of the zygoma is situated above the first or second upper molar and the mental foramen is below the posterior edge of the second lower premolar. In general, the muscular impressions on the skull are extremely well marked,

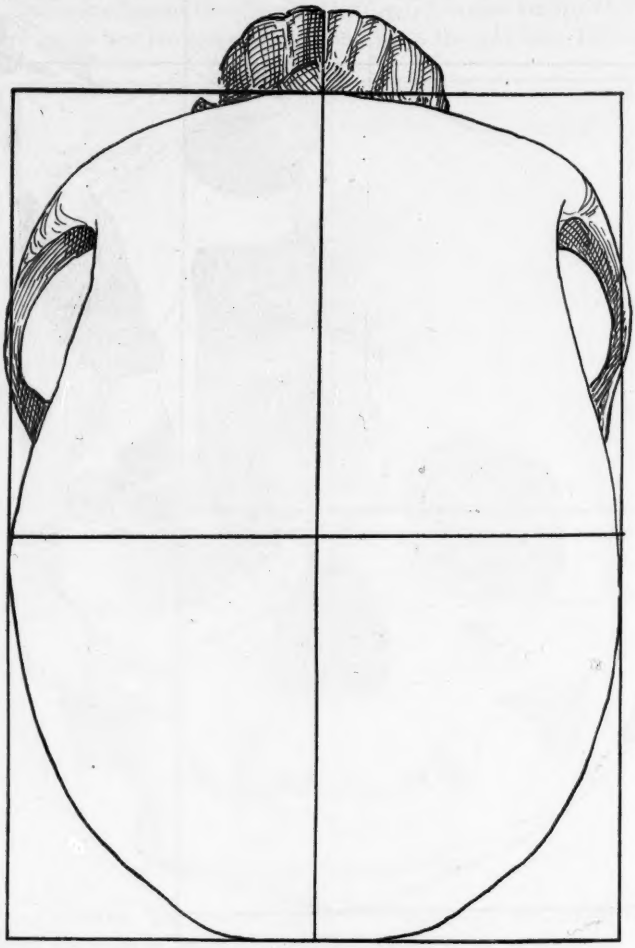

Fig. 3. Norma Verticalis. Benabena. Purari. ♂ A.

and the origin of the temporal is situated high up on the side of the skull. Racially, perhaps the most distinctive features of these skulls are the approach to mesaticephaly without flattening of the occiput: the width of the narial aperture and the marked guttering of its margin, combined with a prominent nasal bridge: the prominence, without great massiveness of the malars: and

the development of the chin in the presence of a high degree of subnasal prognathism. The average cranial capacity is 1280 c.c.

One skull presents the anomaly of almost complete (congenital) absence of nasal bones without any resulting deformity of the nasal bridge.

The smallness of the number in the series, and the accompanying diopterographic drawings of a typical skull, do away with the necessity for printing tables of the metrical and non-metrical details of the skulls.

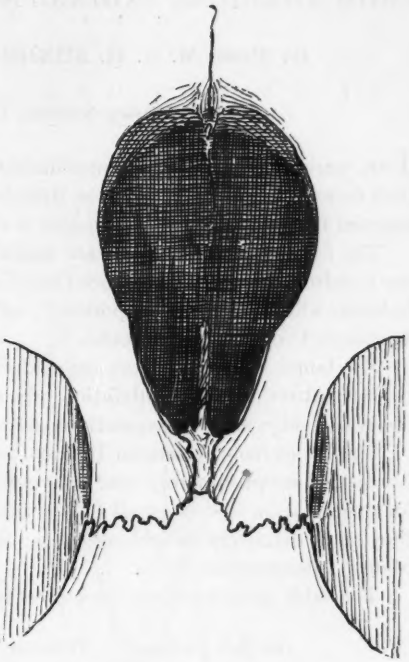

Fig. 4. Case of almost complete (congenital) absence of the nasal bones. Purari. ♂ D.

These people, living as they do upon a high inland plateau, are probably somewhat lighter in colour than the dwellers in the lower coast lands and foothills; but to describe them as a white race is a misuse of well-defined terms. To assert that they are of the Polynesian race, on the evidence of some cultural features, is entirely unwarranted. They are obviously of a curly to frizzy haired, frizzy bearded and deeply pigmented race; with comparatively short heads and wide spread noses with fairly prominent bridges. Undoubtedly they have specialised during their isolated tenure of the high valleys of the Purari watershed, but the specialisation has been social and cultural rather than physical, as physical characters are expressed in cranial features. It is safe to say that the Purari tribes do not differ physically in any material way from other well-known tribes in New Guinea.

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OBSERVATIONS ON THE LOWER END OF THE FEMUR FROM AMERICAN INDIANS AND MODERN CANADIANS

By Prof. M. A. H. SIDDIQI, M.S., F.R.C.S. (Eng.)

Vincent Massey Scholar, University of Toronto

This work has been done on lines similar to that of Martin's investigation on Irish femora (1) and of the writer on British Indian femora (2). For the technique adopted for measurements the reader is referred to these articles.

The following observations are based on an investigation carried out on one hundred femora from modern Canadians and ten from primitive American Indians, which I had the opportunity of investigating in the Department of Anatomy, University of Toronto.

The bones from Canadians are modern and are expected to show the particular feature under consideration of a non-squatter, i.e. a prominent intercondyloid ridge and consequently a shallower intercondyloid fossa.

The bones from American Indians, on the other hand, are from an excavation and are presumably over 400 years old. Although the number available for examination is very small I have incorporated them into the account, as they show definitely the characteristic feature of a squatter; i.e. the presence of deep intercondylar fossa.

The table gives comparative curves for the four types of bones examined:

British Indian:	Total number examined	198
American Indian:	,,	10
Modern Irish:	,,	63 (Martin)
Modern Canadian:	,,	100

The average mean of the ratio $\frac{\text{Antero-posterior depth of articular surface}}{\text{Depth of intercondyloid fossa}}$ for the four groups of individuals is as follows:

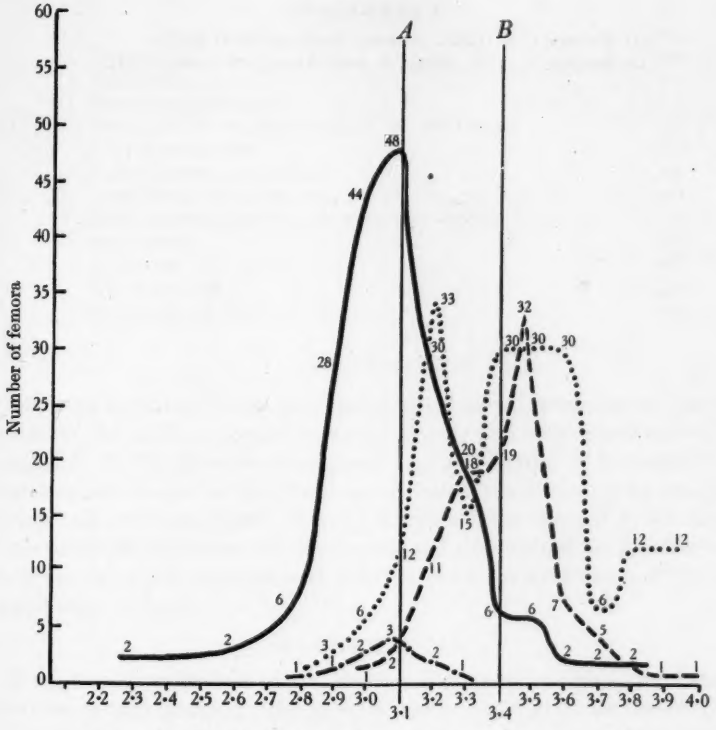
British Indian	3·1	indicated on chart by the vertical line *A*.
American Indian	3·07	
Modern Irish	3·43	,, ,, *B*.
Modern Canadian	3·42	

It will be noticed that the British and the American Indians show deeper intercondyloid fossae than the modern Irish and Canadians, a feature which has been explained in previous articles on the basis of the squatting or non-squatting postures used by these groups of individuals.

Table I

Subjects and total number examined	Number of femora under different ratios																	
	2·3	2·4	2·5	2·6	2·7	2·8	2·9	3·0	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	4·0
British Indian, 198	2	.	.	2	.	6	28	44	48	30	20	6	6	2	2	2	.	.
American Indian, 10	.	.	.	.	.	1	1	2	3	2	1	.	.	.	.	.	.	.
Modern Irish, 189	.	.	.	.	.	.	3	6	12	33	15	30	30	30	6	12	.	12
Modern Canadian, 100	.	.	.	.	.	.	.	1	2	11	19	19	32	7	5	2	1	1

Table II



Number by which the depth of fossa is to be multiplied to equal anterior-posterior depth of articular surface.

——— British Indian (Lucknow)
•–•–•–• American Indian (Toronto)
••••••••• Modern Irish (Martin)
– – – – – – – Modern Canadian (Toronto)

SUMMARY

Observations on the lower end of the femora from 100 Canadians and 10 American Indians have been made and compared with similar observations on 198 British Indians and 63 modern Irish. The conclusion arrived at is that the individuals who use a squatting posture, i.e. British and American Indians, have deeper intercondyloid fossa than those who use non-squatting posture, i.e. modern Irish and Canadian.

I wish to thank Prof. J. C. B. Grant for the facilities and his keen personal interest in the work.

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THE STRUCTURE AND DEVELOPMENT OF THE URETHRAL SINUS IN THE MALE WHITE MOUSE, WITH NOTES ON ITS OCCURRENCE IN OTHER RODENTS

By KATHLEEN HALL

From the Department of Zoology, University College of North Wales, Bangor

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I. INTRODUCTION

It has been known for some time that a glandular diverticulum of the male urethra in the bulb is present in many rodents and some insectivores. Although this diverticulum has been mentioned and figured by several authors, there does not appear to have been any detailed description of its structure, development and homologies. It was felt that a fuller account was desirable. In this paper the structure and development of the urethral sinus in the white mouse are described, together with brief notes on the occurrence of the sinus in some other rodents.

II. LITERATURE

A sac-like diverticulum of the urethra was first discovered by Tullberg (1899) in certain rodents. He gave it the name "sinus urethralis", and apparently did not recognise its glandular nature. Stutzmann (1899) did not mention it. Grosz (1905) found in *Mus* a sinus-like structure given off from the urethra in the neighbourhood of the bulb, near the openings of the ducts of Cowper's glands. He commented upon the fact that Tullberg saw this and called it a urethral sinus. Rauther (1909) described a wide pouch, the "sinus urethralis", embedded in the muscle of the bulbar region in several rodents and richly provided with urethral glands. He noted that in a young example

of *Otomys* these glands were particularly well developed on the ventral side, and that Cowper's glands opened near the junction of the sinus and urethra. Rowlands (1936) found a glandular diverticulum of the urethra in the bulb in *Evotomys*.

As regards animals other than rodents, Rauther (1909) found a urethral sinus in *Orycteropus* and several insectivores, including *Macroscelides* and *Sorex*. Kaudern (1910), working on insectivores and lemurs, described a sac-like diverticulum, the so-called "sinus urethralis", opening into the urethra near the openings of Cowper's ducts in several insectivores, including *Centetes*, *Oryzoryctes*, *Potamogale*, *Macroscelides*, *Crocidura* and *Tupaia*, but does not mention it in lemurs. He suggests in passing that the sinus may have arisen during the separation of the urethra and rectum in the embryo, but does not put forward any evidence in support of this view. Grosz (1905) described in the Sciuridae on the dorsal side of the urethra a long duct, the anterior part of which is divided into two by a septum, and the wall of which is glandular. Mossman, Lawlah and Bradley (1932), in a paper on the male reproductive tract of the Sciuridae, described a structure, which they called the "bulbar gland", connected by a long "penile duct" to the urethra near the flexure of the penis. This was found to be absent in *Tamiasciurus*, where there occurs a "large, non-glandular, ventral urethral diverticulum or sinus in the bulb (sinus urethrae bulbi)", which is absent in other species. In view of the findings in this paper it seems doubtful if the "bulbar gland" of Mossman, Lawlah and Bradley is homologous with the true urethral sinus.

III. MATERIAL AND TECHNIQUE

The material consisted of a number of adult male white mice and immature animals 6 hours, 18 hours, 3, 6, 12, 20 and 26 days old, and the following series of embryos:

Stage	Age	No. of examples	No. of litters
I	15 days post coitum	5	3
II	16 ,,	12	5
III	16 ,,	1	1
IV	16½ ,,	1	1
V	17 ,,	3	3
VI	18 ,,	2	2

Adults of the following species of rodents were examined also: *Apodemus sylvaticus* (Linn.), *Evotomys glareolus* (Schreber), *Microtus hirtus* (Bellamy), *Mus rattus* (Linn.), *Cricetus auratus* (Waterhouse), *Fiber zibethicus* (Cuv.), and *Cavia cobaya* (Schreber).

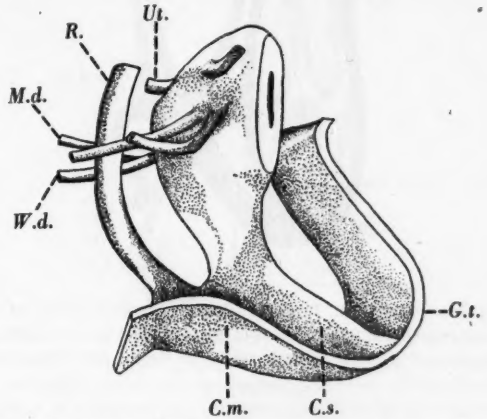
The fixative normally employed was either Bouin's fluid or the alcoholic modification of it. Serial sections of the adult material were cut at 10 μ and of the embryos at 7 μ. The sections were stained with Ehrlich's haematoxylin and eosin. The bulbar regions of two animals were fixed in corrosive sublimate, subsequently washed with 70 per cent. alcohol to which had been added a

few crystals of iodine, and stained with specific mucus stains—methylene blue, toluidine blue, mucicarmine, and mucihaematein—in an attempt to determine the nature of the secretion from the glandular tissue. Graphic reconstructions and wax-plate models were made.

IV. DESCRIPTION OF THE URETHRAL SINUS OF THE WHITE MOUSE

(*a*) *Development*

Stage I (text-figs. 1 and 2). The genital tubercle (*G.t.*) is easily recognisable as a somewhat rounded elevation of the abdominal wall, occupying almost the whole of the space between the umbilical cord and the base of the tail. The cloacal membrane (*C.m.*) extends along the caudal face of the tubercle. The urogenital sinus has the form of a short, straight tube, the base of which is somewhat expanded to form the cloaca (*Cl.*) ("Caudalkammer" of Fleischmann (1902) and his pupils). The rectum and urogenital sinus are separated

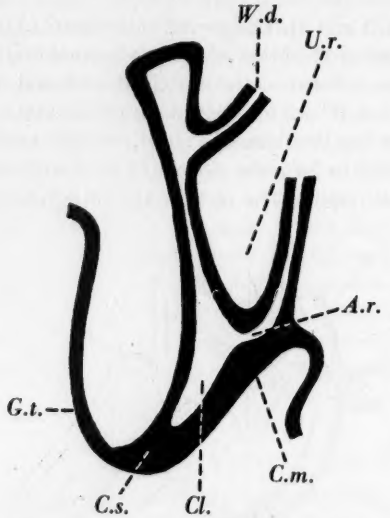


Text-fig. 1. 15-day embryo, stage I. Wax-plate reconstruction of the cloacal region. The cloacal membrane (*C.m.*) is intact, and the cloacal septum (*C.s.*) extends to the tip of the genital tubercle. The latter is shown in section. (See p. 428 for key to guide letters to all text-figures.) ×65.

almost to their bases by the urorectal fold (*U.r.*) into which a narrow prolongation of the coelom extends. A very narrow passage (the "analrohr" of Fleischmann (1903–4), Schwarztrauber (1903–4) and others) connects the hinder end of the rectum with the cloaca.

The cavity of the cloaca is drawn out into the tubercle and lies beneath the cloacal membrane. Distal to it a solid block of cells (*C.s.*) continuous with the ectoderm of the tubercle extends to the tip of the latter (phallusleiste, kloakenplatte, cloison cloacal, uralplatte, cloacal septum, and urethral plate of other authors). The term "cloacal septum" first proposed by Reichel (*vide*

Born, 1893) and adopted by Buchanan and Fraser (1918) will be used here. It has been described by Fleischmann (1902) and his pupils in various Mammals, Felix (1912) in the human embryo, and Buchanan and Fraser (1918) in Marsupials. It is difficult at this stage to determine whether the cloacal membrane has ruptured. In one embryo, very close to the rectal-urogenital connection, there appears to be an extremely minute opening to the exterior, but in the others the membrane is intact, although thinner at this spot.

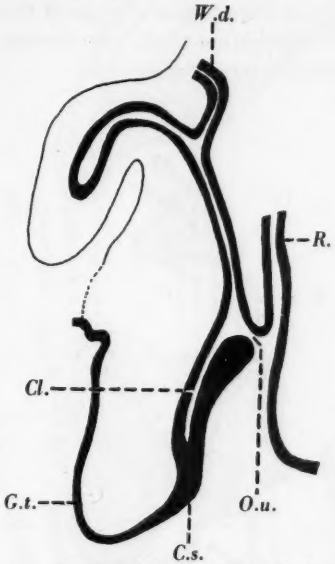


Text-fig. 2. 15-day embryo, stage I. Reconstructed longitudinal section of the cloacal region, showing the expanded base of the urogenital sinus forming the cloaca (*Cl.*), the ventral part of which is drawn out into the tubercle while the dorsal part forms the rectal-urogenital connection (*A.r.*). ×65.

Stage II (text-fig. 3). There is some variation in the embryos comprising this stage. The genital tubercle has increased in size, and in some embryos the base of the tubercle is quite clearly marked off from the tip (or glans) by a well-defined groove, the coronary sulcus. The urorectal fold has grown backwards to the original level of the cloacal membrane, which has ruptured. As a result the connection ("analrohr") between the rectum and urogenital sinus has completely disappeared, and both open by separate apertures to the exterior near the base of the tail.

The cloacal septum (*C.s.*) has increased in length, correlated with the growth of the tubercle. It still contains an extension of the cavity of the cloaca in its proximal part. Fleischmann (1902), Schwarztrauber (1903–4), Felix (1912) and Buchanan and Fraser (1918) believe the septum to be of entodermal origin. According to these authors it is formed by the approxi-

mation and fusion of the lateral walls of the cloaca to form a solid block of entoderm. This fusion commences at the distal end and only gradually proceeds in a proximal direction so that the septum is not completed until a comparatively late stage in development. Our material supports this interpretation, rather than the view of Retterer (1890) and Born (1893) that the septum arises from ectodermal folds. The former theory would account for the extension of the cloacal cavity in the tubercle in some 16-day embryos after the rupture of the membrane.

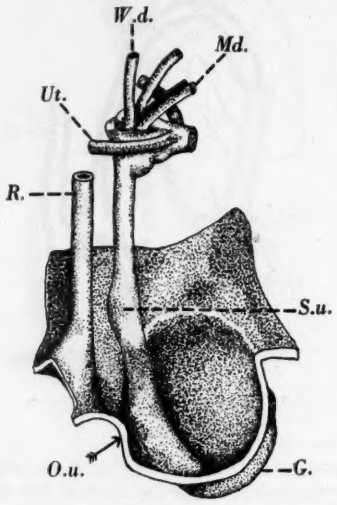


Text-fig. 3. 16-day embryo, stage II. Reconstructed longitudinal section of the cloacal region. The cloacal membrane has ruptured, and the urorectal fold has grown backwards so that the rectum and urogenital sinus open by separate apertures to the exterior. The ventral portion of the cloaca is continued into the tubercle, and distal to it can be seen the solid cloacal septum (*C.s.*). ×32.

Stage III (text-fig. 4). This 16-day embryo is considerably more advanced than the others and constitutes a separate stage. The original urogenital opening near the anus has closed up, and the new aperture (*O.u.*) is situated immediately proximal to the sulcus. The cavity of the urethra is continuous as far as the external opening, the new portion within the tubercle having been formed by the fusion of a pair of genital folds which have grown up on either side of the cloacal septum. Fusion of these folds commences at the proximal end in the neighbourhood of the original urogenital aperture. In stage III the folds have fused almost as far as the coronary sulcus; beyond the urogenital aperture they are continued for a very short distance. At the base of the tubercle and close to the anus, there is a dorso-lateral dilation of

the lumen at what appears to be the junction of the primary and secondary portions of the urethra (text-fig. 4, *S.u.*).

Stage IV. In this embryo, the urethral dilation has increased in size. It is situated on the rectal or caudal side at the point where there is a slight flexure of the urethra. Comparing this stage with the last, the chief difference lies in the relative growth of the different parts. The tubercle has increased in length, especially the basal part between the sulcus and the anus, so that the penile urethra is longer. Immediately distal to the urogenital aperture is a solid block of cells representing all that remains of the cloacal septum. There is a certain amount of organisation of the mesodermal tissue adjacent to the urethra, and erectile tissue is now recognisable.

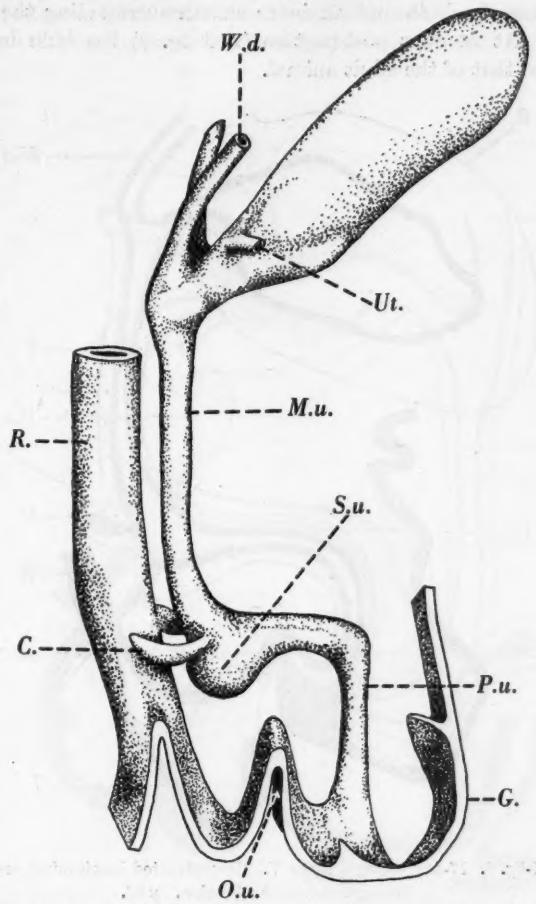


Text-fig. 4. 16-day embryo, stage III. Wax-plate reconstruction of the posterior part of the urogenital system, showing the rudiment of the urethral sinus (*S.u.*). The penile urethra opens, as indicated by the arrow, at the coronary sulcus. ×32.

Stage V (text-figs. 5 and 6). Development during the next 12 hours consists in further differentiation and organisation of the structures already formed. The urogenital aperture (*O.u.*) is still situated at the sulcus; the glans has become more rounded. Erectile and muscular tissues are better developed. The rudiments of Cowper's glands are represented by two small, solid, club-like structures (*C.*) which appear to have arisen as bud-like outgrowths from the urethral epithelium, and which are situated just where the urethral lumen is dilated. The position of these glands provides strong evidence that the dilation is the rudiment of the urethral sinus of the adult.

Stage VI (text-fig. 7). The urogenital aperture has shifted from the coronary sulcus to the tip of the glans. Organisation of the muscular and erectile tissue

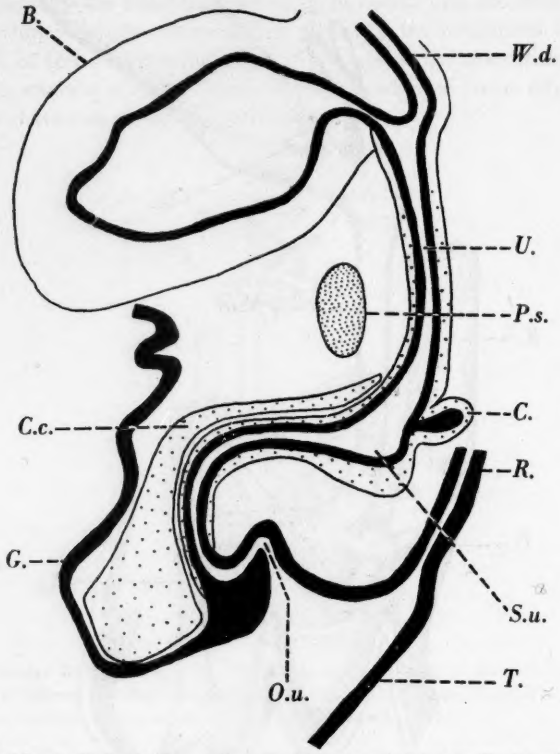
has proceeded, and minute simple glands have appeared in the part of the urethra proximal to the bladder, but the rest of the wall is non-glandular. Cowper's glands have acquired cavities.



Text-fig. 5. 17-day embryo, stage V. Wax-plate reconstruction of the posterior part of the urogenital system. Cowper's glands are situated in the neighbourhood of the urethral sinus. The penile urethra opens immediately distal to the coronary sulcus. × 32.

At parturition the cavity of the sinus, instead of being rounded, is narrow and peg-like, and 100 μ in length. There are a few very small and simple glands in the wall of the urethra and sinus. Cowper's glands are larger, with more conspicuous cavities and short ducts which open into the sinus. The erectile tissue of the corpus spongiosum and corpus cavernosum is not yet fully vascularised. During the next month all the adult structures become

fully differentiated; the sinus and associated structures increase in size, the glandular tissue becomes more compact, the erectile tissue completely vascularised, and the muscular tissue is divided into an outer sheath forming the greater part of the bulb and an inner sheath surrounding the sinus itself (text-fig. 8). At 26 days post-partum (text-fig. 9) the bulb has a similar appearance to that of the adult animal.

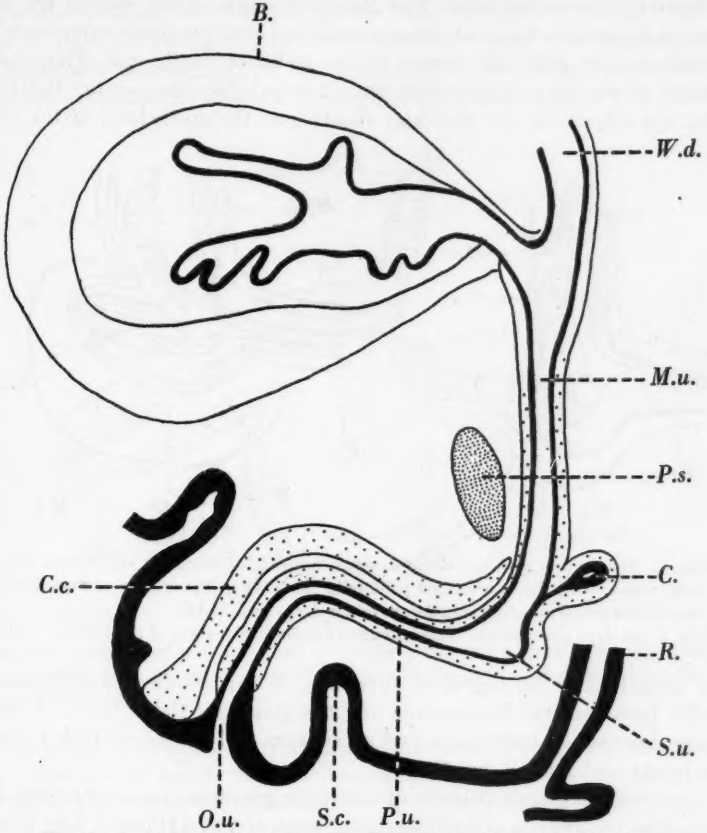


Text-fig. 6. 17-day embryo, stage V. Reconstructed longitudinal section through the urethral region. ×32.

(*b*) *Anatomy and histology*

The urethral sinus in the adult white mouse is a large sac-like glandular diverticulum (text-figs. 10, 11, 12, *S.u.*), completely surrounded by, and closely attached to, the musculature of the bulb. This sinus varies in shape and size according to the degree of distension and the amount of secretion in the lumen. It is sometimes long and narrow, with a cleft-like lumen and a thick wall, or more or less rounded in shape, with a large cavity and a comparatively thin wall (compare text-fig. 11 with Plate I, fig. 1). The tip of the sinus often

bifurcates, but the bifurcation is less marked in the white mouse than in certain other rodents. The bulbo-urethral (Cowper's) glands lie outside the musculature of the bulb. Their ducts (Plate I, fig. 1, *C.d.*) pass inwards and run obliquely through the muscle and erectile tissue to open into the sinus (text-fig. 11, *C.d.*) very close to its opening into the urethra.

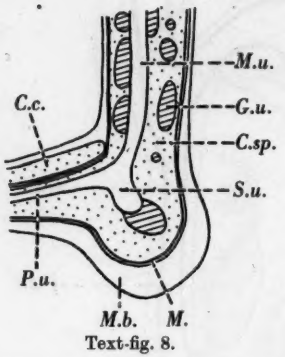


Text-fig. 7. 18-day embryo, stage VI. Reconstructed longitudinal section through the urethral region. ×32.

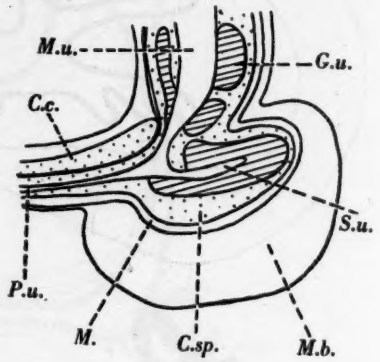
The outer muscular sheath (Plate I, fig. 1) which composes the greater part of the bulb, and which encloses the urethra and sinus, is made up entirely of striped fibres. Enclosing the sinus within the striped muscle is a thick outer sheath of fibrous connective tissue (*C.t.*) and an inner of smooth muscle (*M.*) which is very imperfectly divided into two layers. Septa or trabeculae of smooth muscle pass inwards from this latter sheath between the cavernous spaces of the erectile tissue of the corpus spongiosum, which,

with the glandular tissue (*G.u.*), comprises the greater part of the wall of the sinus. The wall of the sinus, therefore, has essentially a similar structure to that of the urethra.

The urethra is usually glandular where it enters the bulb and as far as the opening into it of the sinus (text-figs. 10 and 12). The penile urethra distal to the sinus is non-glandular. The glandular tissue in the wall of the sinus always is better developed at the proximal end near the pubic symphysis and laterally, and is generally absent at the distal or penile end. The relative amounts of erectile and glandular tissues, which together occupy the whole of the space between the muscular sheath and the epithelium of the sinus,



Text-fig. 8.



Text-fig. 9.

Text-fig. 8. 20-days post-partum. Reconstructed longitudinal section of the bulbus. The glandular tissue (*G.u.*) lies in scattered patches in the walls of the urethra and sinus, and the erectile tissue and muscular sheaths are better developed. ×16.

Text-fig. 9. 26-days post-partum. Reconstructed longitudinal section of the bulbus. ×16.

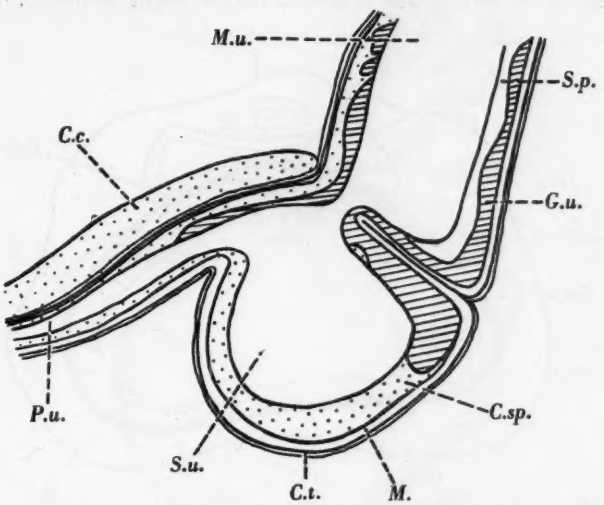
vary according to the degree of distension. When the sinus is distended the erectile tissue is very hyperaemic and the glandular tissue lies in scattered lobules against the epithelium and so appears to be small in bulk (compare text-fig. 11 and Plate I, fig. 1).

The urethral glands (Glands of Littré, or glandulae paraurethrales) lie in the wall of the urethra and of the sinus. Each alveolus (Plate I, figs. 2 and 3) is very small, and in each section is made up of six to eight wedge-shaped cells, the nuclei of which are oval and lie near the bases of the cells. The lumen of the alveolus is extremely small and often difficult to distinguish. The protoplasm of the alveolar cells stains rather darkly with haematoxylin and each contains a secretory vacuole. The alveoli open into very small ducts lined by cubical, darkly staining cells. These unite to form larger ducts which open into collecting ducts with very low epithelium, flattened nuclei and wide lumina. The collecting ducts open into the cavity of the sinus or urethra.

The epithelium of the urethral sinus, and of the urethra in the neighbour-

hood of the bulb, is transitional in character, the nuclei being arranged in one to three rows.

The bulbo-urethral (Cowper's) glands are enclosed within a sheath of fibrous connective tissue inside which is a layer of striped muscle; not smooth, as in the urethra and sinus. The glandular tissue (Plate I, fig. 4) differs markedly in appearance from the urethral glands. The tubules are lined with tall columnar cells, the protoplasm of which stains less deeply with haematoxylin. The nuclei are flattened against the basal walls of the cells. The alveoli are larger than those of the urethral glands, and have more conspicuous lumina.



Text-fig. 10. Longitudinal section through the urethral sinus and adjacent parts of the urethra of an adult white mouse. The penile urethra and the distal part of the sinus are non-glandular. × 16.

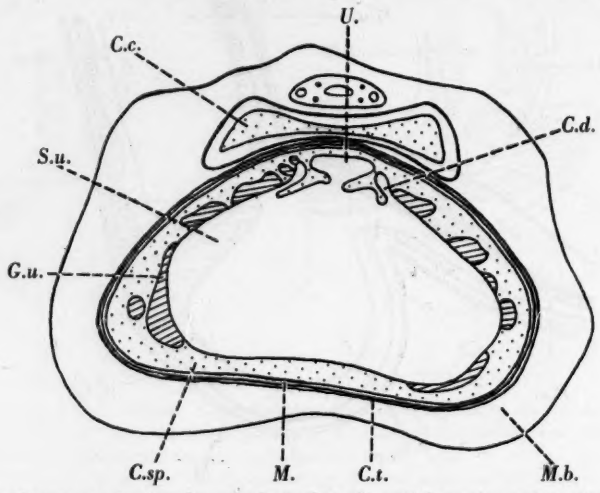
Strands of muscular and connective tissue pass inwards between the tubules, which drain into a cavity more or less centrally situated in the gland and continuous with the lumen of the duct.

The walls of the ducts of Cowper's glands (Plate I, fig. 5) are composed of two kinds of tissue, some of the cells being similar in appearance to those composing the gland itself, while others, more darkly staining, and in very small alveoli, resemble those of the urethral glands. The cells of these alveoli are smaller and their rounded nuclei therefore appear more closely crowded together. The part of the duct nearest to the urethra is usually made up entirely of the latter type of tissue, but in a transverse section through any other region of the duct both kinds of tissue are usually present sharply marked off from one another. In the ducts of the bulbo-urethral glands of some animals there can be seen, in material fixed in Bouin's fluid, oval bodies

which appear to lie within the cells of the more darkly staining alveoli (Plate I, fig. 5). These bodies are fibrillar in structure, stain deeply with eosin, and are highly refractive in appearance. They nearly fill the cells in which they are found, so that the nuclei are pushed to the side and often distorted. It is probable that these bodies are composed of substances about to be secreted.

(*c*) *Nature of the secretion*

An attempt was made to determine the nature of the substance secreted by the glandular tissue of the urethral sinus and Cowper's glands. From the appearance of the cells it was suspected that it might be mucin. After the



Text-fig. 11. Transverse section through the bulbus of an adult at the position of the opening of the ducts of Cowper's glands into the urethral sinus. The sinus is very much distended, and the glandular tissue lies in scattered patches against the epithelium. × 16.

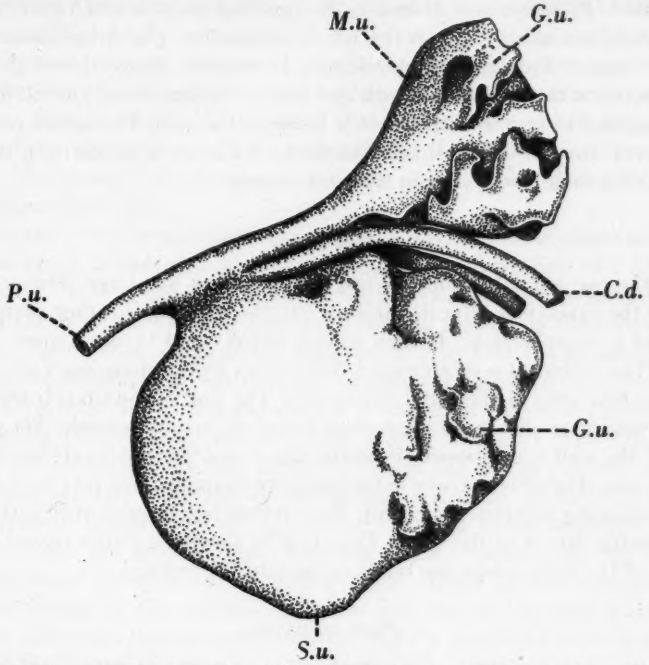
use of specific mucus stains, Cowper's glands gave a positive reaction and the glands of the urethra and sinus a negative reaction. Cowper's glands stained particularly brilliantly with mucicarmine and mucihaematein. Those parts of their ducts which were composed of tissue resembling that of the body of the gland took up the stain while the other parts did not.

Disselhorst (1904) declares that he has never obtained a definite mucus reaction in Cowper's glands, and maintains that the secretion of these glands cannot be mucus, as no precipitation was obtained on the addition of acetic acid. He quotes Müller as having obtained a mucus reaction in Cowper's glands after staining with thionin, but does not attach much significance to this.

It is only possible to state here, that after using various specific mucus stains Cowper's glands gave a positive and the glands of the urethra and sinus a negative result.

V. NOTES ON THE URETHRAL SINUS IN SOME OTHER RODENTS

A sinus urethralis bulbi has already been discovered and figured in several rodents. Tullberg (1899) found it in *Anomalurus*, *Dipus*, *Mus decumanus*, and *Haplodon rufus*. Grosz (1905) noted it as being present in *Mus sylvaticus* (=*Apodemus sylvaticus* of this paper) and *Dipus*, and Rauther (1909) in *Otomys*, *Arvicanthus* and *Mus*. Mossman, Lawlah and Bradley (1932) found a urethral sinus in *Tamiasciurus*, but described it as being non-glandular.



Text-fig. 12. Wax-plate reconstruction of the urethral sinus and associated structures in a distended condition. The outer limits of the epithelium and glandular tissue are shown. ×20.

No attempt is made here to provide an exhaustive list of rodents possessing a true urethral sinus. Of the seven species examined, a sinus was found to be present in *Evotomys glareolus*, *Microtus hirtus*, *Apodemus sylvaticus*, *Mus rattus*, *Cricetus auratus*, and *Fiber zibethicus*, but appeared to be absent in *Cavia cobaya*.

Cricetus auratus

In the golden hamster, the sinus is less conspicuous than in the mouse. It is represented by a dilation of the urethral lumen, in the walls of which the glandular tissue is thicker and better developed than in the urethra. The ducts of Cowper's glands are wide, and their walls glandular throughout their

length. They open into the urethra proximal to the sinus, thus differing from the mouse in which the ducts of Cowper's glands open into the sinus itself.

The tissue composing the walls of the ducts of Cowper's glands resembles the glandular tissue in the urethral wall in appearance. The wall of the penile urethra is glandular for some distance distal to the sinus.

Evotomys glareolus and *Microtus hirtus*

In both *Evotomys* and *Microtus*, the urethral sinus is much more deeply bifurcated proximally than in the white mouse. The glandular tissue in the wall is compact and very well developed. It occupies almost the whole of the space between the muscular sheath and the epithelium lining the cavity, and there appears to be very little erectile tissue in the wall. The actual cavity of the sinus is comparatively small. The ducts of Cowper's glands open into the sinus in the same position as in the white mouse.

Mus rattus

In the one animal examined, the urethral sinus was large. The proximal part of the sinus, just after its opening into the urethra, is rather narrow and rounded in cross-section. It then widens distally and becomes more oval in shape. The extreme tip is bifurcated. The cavity is of medium size and extends into the two lobes at the distal bifurcation. The wall of the sinus is thick and is surrounded by sheaths of connective tissue and smooth muscle. The greater part of the wall is composed of erectile tissue and scattered patches of glandular tissue. The erectile tissue in the specimen examined was very hyperaemic, thus increasing its apparent extent. The urethra is glandular until just before the opening into it of the sinus. The ducts of Cowper's glands open into the cavity of the sinus where the latter opens into the urethra.

Fiber zibethicus

In the musk rat (Plate I, fig. 6) the glandular tissue of the urethral sinus is the most conspicuous feature in the bulb. The cavity of the urethra is relatively very small and slit-like with a folded epithelium of clear columnar cells with oval basal nuclei, beneath which are two or three rows of closely packed nuclei, and a thin layer of cavernous erectile tissue. The whole of the centre part of the bulb is occupied by an enormous mass of compact alveolar glandular tissue. A sheath of fibrous connective tissue and a thin layer of smooth muscle enclose the glandular tissue and are continuous around the urethra. The cavity of the urethral sinus is extremely small and cleft-like. It is bounded by an epithelium which is continuous with and similar in structure to that of the urethra. The erectile tissue, which is small in amount, is chiefly confined to the distal part of the sinus. The ducts of Cowper's glands open into the cleft-like cavity of the urethra.

VI. CONCLUSIONS

In the males of some mammals, in particular certain rodents and insectivores, the urethra upon entering the bulb gives off a blind sac-like diverticulum, the urethral sinus. The appearance of this sinus in the white mouse is typical of most of the animals examined. Its wall is continuous with and similar in structure to that of the urethra itself, and the ducts of Cowper's glands open into its base. Glands, resembling those of the urethra, but differing from the tissue composing Cowper's glands, are present in the wall of the sinus. The penile urethra distal to the sinus is usually non-glandular. The position of the sinus, situated as it is at the junction of the muscular and penile portions of the urethra in the neighbourhood of the pubic symphysis and quite close to the anus, suggested that it may have arisen during the closure of the primitive urogenital aperture and the formation of the penile urethra. The changes which take place in the cloacal region during development support this view.

In the early 16-day embryos (stage II) the urogenital sinus passes straight back and opens to the exterior near the anus; there is no sign of a dilation of its cavity. During the next 12 hours the genital folds, which have grown up on either side of the cloacal septum, meet and fuse along the face of the tubercle. Fusion commences at the proximal end, so that the primitive urogenital aperture closes up, and the new aperture is shifted distally towards the tip of the tubercle. In this way the penile urethra is formed as a secondary structure, its lumen being continuous with that of the upper part. The rudiment of the urethral sinus first appears in stage III at the junction of the primary and secondary portions of the urethra, and there can be little doubt that it represents that part of the primary urethra which originally communicated with the exterior, but which has become closed in by the fusion of the proximal portions of the genital folds. Later in development the muscular sheaths of the urethra and sinus and the erectile and glandular tissues in their walls become differentiated, and the ducts of Cowper's glands open into the base of the sinus.

The author's thanks are due to Prof. F. W. R. Brambell, for advice and criticism, and to Dr A. S. Parkes, F.R.S., for providing the musk rat and golden hamster material.

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KEY TO LETTERING

A.r.	Rectal-urogenital connection.	*M.d.*	Müllerian duct.
B.	Bladder.	*M.u.*	Muscular urethra.
C.	Cowper's gland.	*O.u.*	Urogenital aperture.
C.c.	Corpus cavernosum.	*P.s.*	Pubic symphysis.
C.d.	Duct of Cowper's gland.	*P.u.*	Penile urethra.
Cl.	Cloaca.	*R.*	Rectum.
C.m.	Cloacal membrane.	*S.c.*	Coronary sulcus.
C.s.	Cloacal septum.	*S.p.*	Shrinkage space.
C.sp.	Corpus spongiosum.	*S.u.*	Urethral sinus.
C.t.	Connective tissue sheath.	*T.*	Tail.
G.	Glans.	*U.*	Urethra.
G.t.	Genital tubercle.	*U.r.*	Urorectal fold.
G.u.	Urethral glands.	*Ut.*	Ureter.
M.	Inner muscular sheath.	*W.d.*	Wolffian duct.
M.b.	Outer musculature of bulb.		

EXPLANATION OF PLATE I

Fig. 1. Photomicrograph of a transverse section through the bulbus of an adult white mouse. ×9.
Fig. 2. Photomicrograph of part of the glandular tissue in the wall of the urethra. ×175.
Fig. 3. Photomicrograph of part of the glandular tissue in the wall of the urethral sinus. ×175.
Fig. 4. Photomicrograph of part of the tissue composing Cowper's gland. ×175.
Fig. 5. Photomicrograph of a transverse section through the duct of Cowper's gland, showing the two kinds of glandular tissue. The fibrillar bodies can be seen among the darkly staining tissue on the right. ×175.
Fig. 6. Photomicrograph of a transverse section through the urethral sinus of the musk rat. ×5.

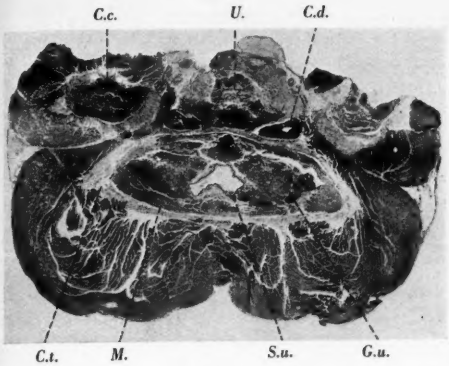
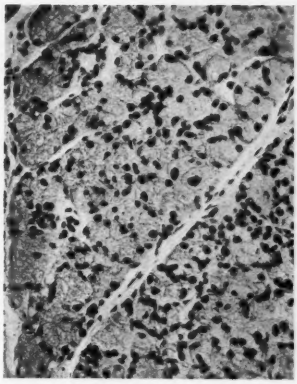


Fig. 1



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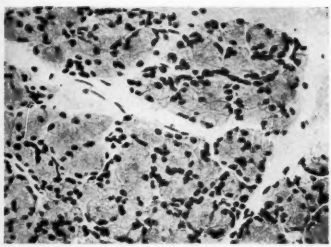

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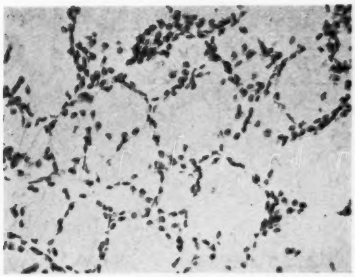

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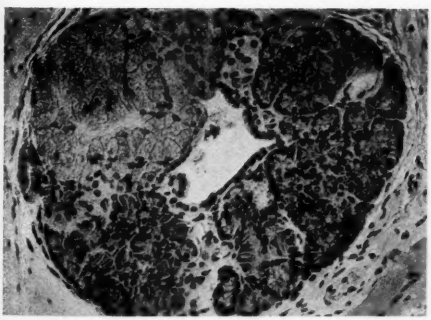

Fig. 5

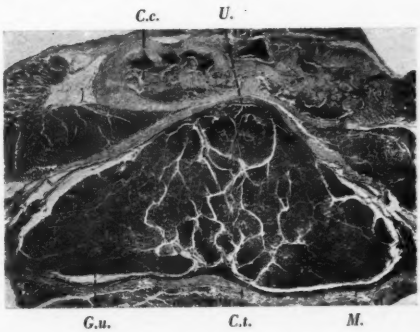

Fig. 6

UNIV. OF MICH.

ABNORMAL PRONUCLEI IN THE FERRET

By WILLIAM J. HAMILTON

The Anatomical Department, St Thomas's Hospital Medical School, London

Abnormal pronuclear ova in Mammals are somewhat rare if we may judge from the number of specimens so far recorded. They have been described by R. van der Stricht (1911) in the cat, by Hill and Tribe (1924) in the cat, by Kremer (1924) in the mouse, and by Mainland (1930) in the ferret.

During my investigation of the "Early stages in the development of the ferret: fertilisation to the formation of the prochordal plate" (1934), I found in the material which I had collected three abnormal pronuclear stages. Two of the specimens were similar to those described by the above observers in that they had three pronuclei. In the third specimen there were four pronuclei present.

DESCRIPTION

Ova Nos. 1 and 2 were obtained from the same ferret at 44½ hours after insemination; there were five other normal pronuclear stages in this ferret. Ovum No. 1 is present in eleven sections of 10 μ in thickness. The cytoplasm has a definite polar arrangement. Two of the pronuclei are of approximately the same size; they are in contact and eccentrically placed (Plate I, figs. 1 and 2). The other pronucleus is smaller and is lying at some distance from these in the deutoplasmic pole of the egg (Plate I, fig. 3). The chromatin granules in all of these pronuclei are well developed and appear to stain normally and are distributed on a definite reticulum. There is no indication in any of these pronuclei to show which is male and which female.

Ovum No. 2 is present in nine sections of 10 μ in thickness; it shows two pronuclei eccentrically placed and in contact and a third pronucleus lying at some distance from these. The polarity of the cytoplasm in this specimen is doubtful. Unlike Ovum No. 1, where the chromatin is well stained, this specimen has the chromatin poorly stained and the reticulum is absent. These pronuclei are apparently undergoing some degenerative change (Plate I, fig. 4); all the pronuclei show the same degree of degeneration.

Ovum No. 3 was obtained from a ferret 41½ hours after insemination and is present in ten sections of 10 μ in thickness. The cytoplasm exhibits a definite polarity. Four pronuclei are present in this specimen, two are lying near the protoplasmic pole but not in contact, whilst two others are lying in contact towards the equator of the egg. All the pronuclei in this specimen are small, the nuclear membranes are poorly defined, the chromatin granules are indistinct and the reticulum is not visible.

COMMENTS AND COMPARISONS

Supernumerary pronuclei do not appear to be very rare in the ferret, since I found three examples in a series of 124 early ova. I have not been able in the ferret to distinguish by their appearance or position the male and female pronuclei (Hamilton, 1934). In the case of the ovum described by R. van der Stricht (1911) in the cat, two of the pronuclei were situated in their normal position at the plastic half of the egg, whilst the third pronucleus was situated some distance from these. In each of the two abnormal ova described by Hill and Tribe (1924) in the cat, the supernumerary pronucleus was stated to be of approximately the same size as the larger of the other pronuclei (which were both eccentrically placed). They believed that the larger pronucleus was the male pronucleus. That supernumerary pronuclei may be the result of fertilisation of binucleated ova was suggested and figured by Kremer (1924) in the mouse. This explanation, however, seems unlikely in the case of the ferret, since Mainland (1930), in the examination of 496 adult ovaries of the ferret, never found a binucleated ovum, nor did I in the examination of many ovaries. Ovum No. 1 described in this paper appears to be very similar to the trinucleated ovum described by Mainland (1930) in the ferret.

Now polyspermy is a normal phenomenon in the ova of Selachians, Reptiles and Birds (Brachet, 1935, and others), and the passage of supernumerary sperms into the ovum gives rise to supernumerary nuclei; but these nuclei soon degenerate, in some cases after abortive attempts to form asters. Further, polyspermy has been described in the mammal *Ornithorhynchus* by Gatenby and Hill (1924), and supernumerary sperms are often found in the perivitelline space of the ovum of the ferret and other Mammals at the early stages of fertilisation (see my former paper for a full description). Thus the occasional occurrence of supernumerary pronuclei may perhaps be attributable to the penetration of the vitelline membrane by more than one sperm.

The fact that all the pronuclei of one of my specimens are showing degenerative changes would seem to indicate that ova showing supernumerary pronuclei do not proceed far in development in the ferret, thus differing from those of animals in which polyspermy is physiological and in which only the supernumerary pronuclei degenerate.

I wish to record my thanks to Prof. A. B. Appleton of St Thomas's Hospital Medical School for his valuable criticism.

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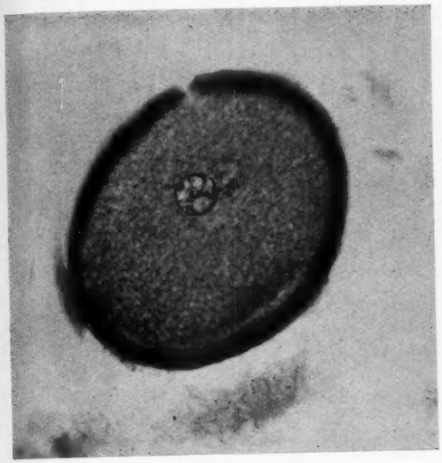

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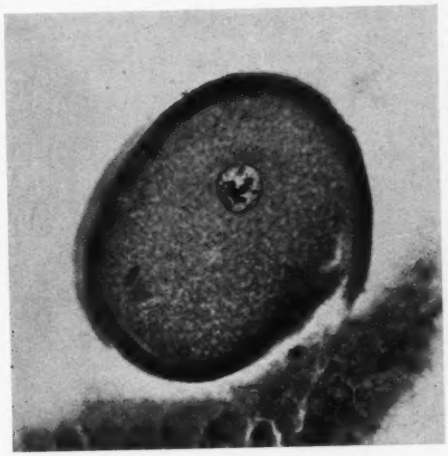

Fig. 2

Fig. 3

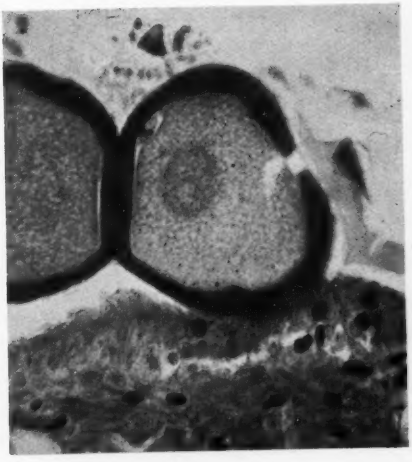

Fig. 4

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EXPLANATION OF PLATE I

Fig. 1. The sixth section of ovum No. 1 showing one complete pronucleus and part of another pronucleus.

Fig. 2. The seventh section of ovum No. 1 showing a pronucleus.

Fig. 3. The tenth section of ovum No. 1 showing the supernumerary pronucleus.

Fig. 4. The seventh section of ovum No. 2 showing two degenerating pronuclei in contact.

THE UNION OF CERTAIN EPIPHYSES IN A MIXED FEMALE POPULATION IN RANGOON

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This inquiry was initiated to establish some age criteria for medico-legal purposes.

101 school girls were examined, the ages ranging between 12 and 22 years. Eighty per cent. of these were pure Burmese and the remainder were of Burmese origin. The ages, although generally translated from Burmese months and years, are assumed to be correct within a month or so. The radiographic method was employed and the upper extremity epiphyses examined.

Table I shows the period of union of these epiphyses as shown in the latest editions of the leading anatomy text-books.

Analysing the times of union obtained in the enquiry we find the following results:

Internal epicondyle

At 12 years in 25 % of cases
13 „ 46·6 % „
14 „ 80·9 % „
15 „ 88·8 % „
16 „ 100 % „
From 16 years onwards 100 % of cases
(Cunningham's figure 14–15 years.)

External epicondyle

At 12 years in 87·5 % of cases
13 „ 93·3 % „
14 „ 90·9 % „
15 „ 100 % „
From 15 years onwards 100 % of cases
(Cunningham's figure 14–15 years.)

Lower end of humerus

At 12 years 100 %; from 12 years onwards 100 % of cases

Olecranon process of ulna

At 12 years in 75 % of cases
13 „ 86·6 % „
14 „ 90·4 % „
15 „ 100 % „
16 „ 94·1 % „
17 „ 100 % „
(Cunningham's figure 14–15 years.)

Head of radius

At 12 years in 50 % of cases
13 „ 73·3 % „
14 „ 85·7 % „
15 „ 94·4 % „
16 „ 100 % „
(Cunningham's figure 14–15 years.)

Lower end of radius

At 12 years in nil
13 „ 6·6 % of cases
14 „ nil
15 „ 33·3 % of cases
16 „ 41·1 % „
17 „ 66·6 % „
18 „ 50 % „
(Cunningham's figure 19–20 years.)

Lower end of ulna

At 12 years in nil
13 „ 13·3 % of cases
14 „ 9·5 % „
15 „ 44·4 % „
16 „ 58·8 % „
17 „ 83·3 % „
18 „ 75 % „
(Cunningham's figure 19–20 years.)

Pisiform (appearance)

At 12 years in 87·5 % of cases
13 „ 100 % „
From 13 years onwards 100 % of cases
(Cunningham's figure 9–10 years.)

Base of first metacarpal

At 12 years in	37·5 %	of cases
13 „	40 %	„
14 „	80·9 %	„
15 „	83·3 %	„
16 „	100 %	„

(Cunningham's figure 17 years.)

Greater tuberosity of humerus

At 12 years in	37·5 %	of cases
13 „	40 %	„
14 „	38 %	„
15 „	72·2 %	„
16 „	82·3 %	„
17 „	75 %	„
18 „	100 %	„

(Cunningham's figure 18 years.)

Lesser tuberosity of humerus

At 12 years in	37·5 %	of cases
13 „	26·6 %	„
14 „	47·6 %	„
15 „	66·6 %	„
16 „	76·4 %	„
17 „	75 %	„
18 „	100 %	„

(Cunningham's figure 18 years.)

Upper end of humerus

At 12 years in	nil	
13 „	20 %	of cases
14 „	4·2 %	„
15 „	33·3 %	„
16 „	29·4 %	„
17 „	33·3 %	„
18 „	50 %	„

(Cunningham's figure 18 years.)

Acromion process of scapula

At 12 years in	nil	
13 „	6·6 %	of cases
14 „	14·2 %	„
15 „	50 %	„
16 „	64·7 %	„
17 „	83·3 %	„
18 „	100 %	„

(Cunningham's figure 20–25 years.)

Coracoid process of scapula

At 12 years in	25 %	of cases
13 „	6·6 %	„
14 „	28·5 %	„
15 „	61·1 %	„
16 „	76·4 %	„
17 „	83·3 %	„
18 „	100 %	„

(Cunningham says at puberty.)

Table I

Secondary centres	Union: Gray	Union: Cunningham	Union: Quain
Elbow:			
(*a*) Internal epicondyle	Unites with the body at the 18th year	M. 18–21; F. 14–15	Unites with the body at the 18th year
(*b*) External epicondyle	Do. 16th–17th year	M. 17–18; F. 14–15	Do. 17th year
(*c*) Lower end of humerus	Do. 18th year	M. 19; F. 14–15	Do. 18th year
(*d*) Olecranon process of ulna	Do. 16th year	M. 18–19; F. 14–15	Do. 17th–19th year
(*e*) Head of radius	Do. 17th or 18th year	M. 18–19; F. 14–15	Do. 17th–19th year
Wrist:			
(*a*) Lower end of radius	Unites with the body at the 20th year	M. 21; F. 19–20	Unites with the body at the 18th–20th year
(*b*) Lower end of ulna	Do. 20th year	M. 21; F. 19–20	Do. 18th–20th year
(*c*) Pisiform	Ossifies at the 12th year	M. 13–14; F. 9–10	Ossifies at the 10th year
(*d*) Base of first metacarpal	Unites with the body at the 20th year	M. 19; F. 17	Unites with the body at the 20th year
Shoulder:			
(*a*) Greater tubercle	Unites with the body at the 20th year	M. 21–; F. 18	Unites with the body at the 20th–22nd year
(*b*) Lesser tubercle	Do. 20th year	M. 21; F. 18	Do. 20th–22nd year
(*c*) Upper end of humerus	Do. 20th year	M. 21; F. 18	Do. 20th–22nd year
(*d*) Acromial end of scapula	Do. 25th year	M. 20–25	Do. 22nd–25th year
(*e*) Coracoid process of scapula	Do. 25th year	Puberty	Do. 15th–18th year

REMARKS AND CONCLUSIONS

The union of female epiphyses occurs earlier than those of the male, and the union of certain epiphyses occurs earlier in a female population of the Burmese races than those of Europeans.

As much reliance has been placed on the humeral epiphyses for determination of age, it is pointed out that the lower end of the humerus may

unite as early as 12 years. The medico-legal significance of this is very great. For instance, abduction of girls and similar crimes are by no means uncommon, as shown by the number of cases sent to X-ray departments for age determination in this connection. If the epiphysis of the lower end of humerus has united we may erroneously assume (on anatomy text-book figures) that the girl is 15 years. If, however, we are in a position to state that the girl is only 12 years then the legal aspect of the crime is completely changed.

Further, the base of the first metacarpal bone united very regularly 1 year earlier, in the above list of females, than western experience led one to expect.

As it is well known that female epiphyses of all races tend to unite earlier than in the male the above figures are not of value in male age determination. These sex differences which may amount to a maximum of 6 years (or more) are very striking. It is therefore anticipated that in certain epiphyses as between an Oriental female and a European male the differences between the unions may amount to 8 years or even more.

Also it is noted that the acromial process unites 2 years earlier than anticipated.

The epiphyses for the internal epicondyle of the humerus unites at 16 years—a striking contrast to that of the lower end of the humerus.

Age determination therefore presents many difficulties as between races and sexes and the less we know about epiphyseal union in such races and sexes, the more uncertain must be our calculation of the probable age of a given person.

It will be seen that Eastern races including the Burmese race come to maturity (as shown by bone growth as well as from other evidence) much earlier than Western races.

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REMNANTS OF THE VENOUS VALVES IN THE ADULT HEART

By N. PAN

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My attention was drawn by Dr M. A. Rahman, Demonstrator of Anatomy, to the presence of valves guarding the orifices of the inferior vena cava and the coronary sinus which resembled the tricuspid and mitral valves in that they were fixed by chordae tendineae. I have never met with this peculiarity in the dissecting room during the past 18 years, and I am unaware of any record of a similar occurrence. The following is a description of the abnormal condition.

The heart is of an adult subject normal in measurement and weight. In the right atrium the valve of the inferior vena cava (Eustachian valve) is an unusually large crescentic fold (fig. 1), the convex margin of which is attached along the lower and greater part of the crista terminalis and to the front of the opening of the inferior vena cava. The concave margin which is usually free is fixed by fine thread-like filaments or chordae tendineae to the atrial septum at the lower limb of the limbus fossa ovalis. The valve of the coronary sinus (Thebesian valve) has the left end of its concave margin similarly fixed by chordae tendineae to the atrial septum close above the point of fixation of the other valve.

DEVELOPMENT OF THE VENOUS VALVES

The right venous valve is formed by an inflection of the myocardium at the sinoatrial junction covered by endocardium (2). When fully developed it is larger than the left venous valve which meets above and below at the superior and inferior fornices. This right venous valve thus has a myocardial basis covered on both surfaces by endocardium; ordinarily this myocardial basis only persists at the convex attached margin of the flap forming the taenia terminalis, and the remaining part of the flap is represented by a duplicature of the endocardium only forming the valves guarding the orifices of the inferior vena cava and the coronary sinus. Sometimes the valve presents a cribriform appearance owing to the degeneration and complete absorption of the flap at places. This cribriform appearance is due to the irregular interlacement of the musculature of the flap; actual perforations occur by degeneration at the meshes between the interlacing strands where the endocardial layers are in apposition whereas the more thickened parts consisting of degenerated muscular strands with a covering of endocardium form the persistent parts of the valve. In the specimen under consideration the myocardial

inflection at the sinoatrial junction was obviously more extensive than is usually the case, resulting in the formation of a right venous valve of a considerably larger size. The formation of the chordae tendineae in this case is probably due to the arrangement of the muscle strands or cords in the body of the valve in such a way that they are loosely spread, being separated by

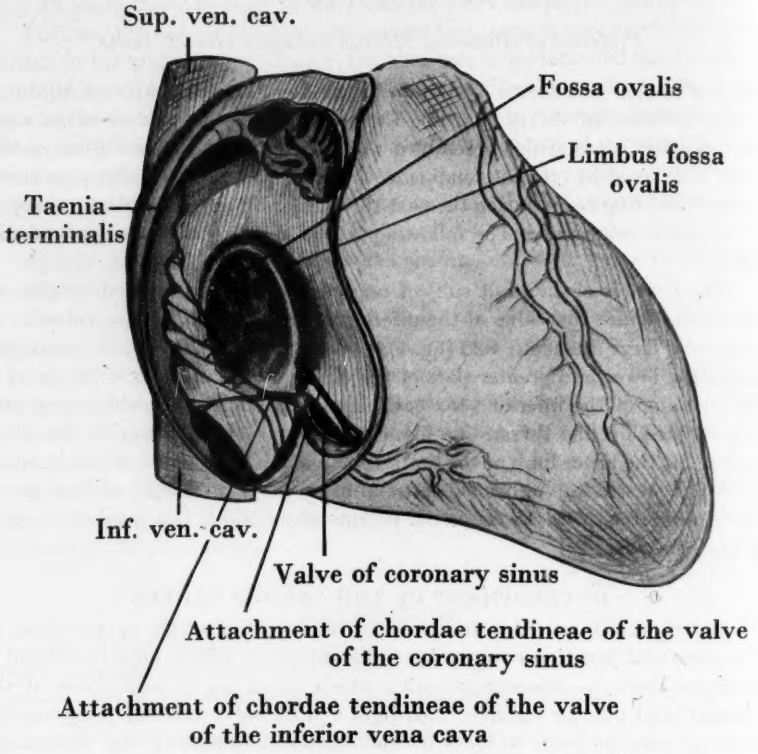
Sup. ven. cav.
Fossa ovalis
Limbus fossa ovalis
Taenia terminalis
Inf. ven. cav.
Valve of coronary sinus
Attachment of chordae tendineae of the valve of the coronary sinus
Attachment of chordae tendineae of the valve of the inferior vena cava

Fig. 1. From original specimen.

greater intervals occupied by endocardium and subendocardial tissue at the cephalic end of the valve and that they converge to a thickened stem at its caudal end. This triangular arrangement of the myocardial strands would explain the formation of the converging chordae tendineae at the caudal end of the flap where they are attached by a thickened stem and diverging cranially are attached to the thickened remains of the body of the flap. This mode of origin perhaps also holds good for the origin of the chordae tendineae in the case of the tricuspid and the mitral valves. There the muscle strands are arranged in such a way that they converge towards the apex of the papillary

muscle and diverge towards their attachment to the flaps of the valves. Where the muscle cords diverge at one end and interlace at the other a combined effect is produced on degeneration of such bundles as is represented in fig. 3.

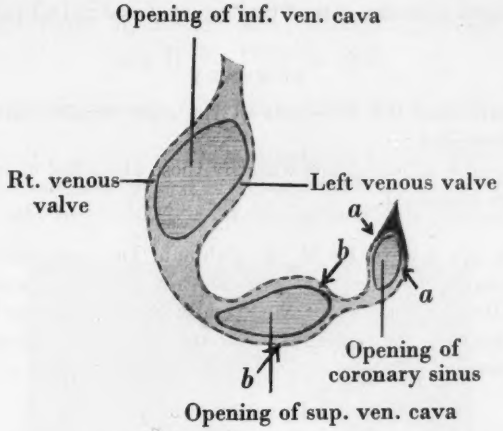

Fig. 2. *aa*. Normal position of fusion of the venous valves. *bb*. Possible abnorma position of fusion of the venous valves in the particular case.

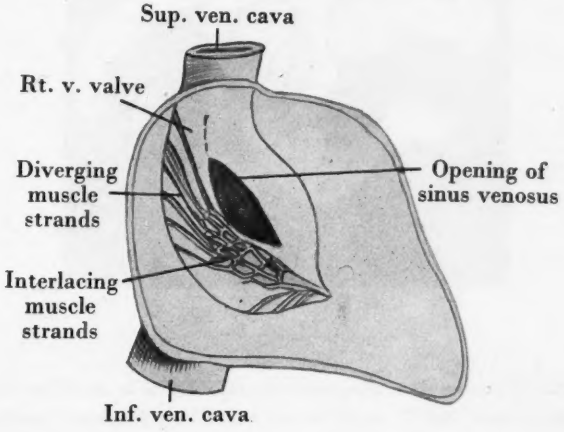

Fig. 3.

The other possibility which may explain the appearance presented in this specimen is this: normally the fusion of the right and left venous valves at the superior fornix is greater than that at the inferior fornix (1). The extent of this normal fusion is shown in fig. 3. In the present case the fusion has

been more extensive at the inferior fornix (fig. 2), consequently the right and left venous valves by their fusion below have produced an extensive fold containing myocardial tissue. Subsequent degeneration of this myocardium from the caudal to the cranial end resulted in the formation of the valve with chordae tendineae as in the case of the tricuspid and mitral valves.

SUMMARY

1. The fixation of the remnants of the right venous valve by chordae tendineae is described.

2. Two possible explanations whereby these abnormal chordae tendineae have arisen are suggested.

My thanks are due to Dr M. A. Rahman, Demonstrator of Anatomy, whose careful scrutiny of the heart specimens revealed this anomaly. I express my thanks to Dr Pasupati Bose, Demonstrator of Anatomy, for kindly making the drawings for me. My grateful thanks are due to Sir Arthur Keith for his valuable suggestions.

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A CASE OF UNUSUAL CONGENITAL MALFORMATION

By Prof. M. N. De, M.B., M.R.C.P. (London)
and H. K. Dutta, M.B.

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Cases of congenital abnormalities associated with maldevelopment of different organs simultaneously in one and the same individual are infrequent. The following case may be of sufficient interest to justify its publication.

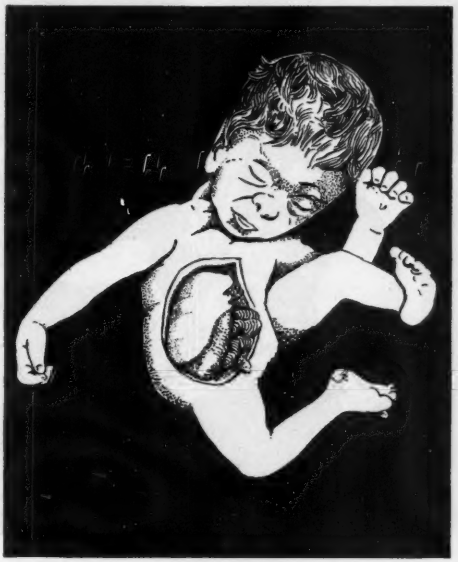


Fig. 1.

The case was that of a still-born foetus apparently about 34–36 weeks old. The general development was below the average. There was marked scoliosis on the right side, the trunk and the lower extremities almost meeting each other at right angles. This bend was occupied on the right side of the abdomen by a bulging produced by the displaced liver and coils of intestine. The upper part of the body presented no peculiarities except that the nipples were absent on the chest wall. In the lower part, however, many abnormalities were seen, both in front and behind.

The outstanding feature was the complete absence of the ventral abdominal wall resulting in the non-closure of the umbilicus and thereby producing a condition of exomphalos. Commencing from the lower part of the epigastric region and extending downwards to the pubis, there was a large inverted "racket"-shaped bare area, the lateral boundaries of which were formed by thickened cord-like folds of peritoneum containing the hypogastric vessels.

Fig. 2.

The abdominal wall on the right side of this gap, extending upwards to the right anterior axillary line, consisted of a thin layer of peritoneum only, the skin and musculature stopping abruptly along this line. The peritoneal covering dipped down at this part to be reflected backwards towards the posterior abdominal wall. On further examination, the floor of the racket-shaped area was found to consist of an upper markedly corrugated part and a lower smooth surface. The coiled intestine when traced caudally was found to terminate abruptly in this corrugated area without apparent further differentiation into large gut and rectum. Histological examination revealed the fact that the coils consisted

of small intestine, while the corrugated area represented the wall of the large gut. There was no external opening of the proctodaeum, the position of the anus being indicated by a dimple in the anal region.

The liver was displaced laterally and forwards and was attached to the posterior abdominal wall by a pedicle containing its blood vessels.

Evidence of external genitalia was entirely lacking. There were two curious finger-like papillae, one on either side, on the medial aspect of the inguinal folds and situated at the extreme lower end of the mammary lines. At first sight each of them looked like a penis. But, on careful examination, these were found to be outgrowths from the skin only, thus probably representing ectopic and abnormal nipples. In a ridge-like body with a bulbous extremity, arising from the smooth area at the lower part of the "racket" and projecting beyond its rounded distal end, rudiments of the prostate gland were found.

The ureters were very tortuous, considerably dilated, and their orifices were found buried in the smooth area at the floor of the "racket", one on either side of the prostatic ridge. This area, therefore, corresponded to the trigonum vesicae.

In the lumbo-sacral region of the back there was a swelling, about the size of a hen's egg, which was found to be a meningocele due to spina bifida of the lower lumbar vertebrae. Both feet showed marked talipes equinovarus. No abnormalities were found in any other organ.

MAIN FEATURES

1. The deficiency of the anterior abdominal wall was associated with concomitant defects of exomphalos, huge ectopia vesicae and arrested development of the external genitalia and urethra.

2. The intestine was not obviously differentiated—there being no apparent large intestine. The cloaca was not separated into dorsal and ventral chambers and the anus was imperforate.

3. Spina bifida.

4. There were two remarkable structures which from their situation are suggested to have been abnormally placed nipples.

RECENT APPOINTMENTS

Prof. J. Kay Jamieson, Dean of the Faculty of Medicine in the University of Leeds, has been appointed Professor of Anatomy and Embryology in the University of Dublin.

Dr C. P. Martin, Chief Demonstrator of Anatomy, Trinity College, Dublin, has been appointed to the Chair of Anatomy in McGill University, Montreal.

REVIEWS

The Study of Anatomy. By S. E. WHITNALL, M.A., M.D., 3rd ed. revised and enlarged. (London: Edward Arnold and Co.) 1936. Pp. 113. Price 4*s.* 6*d.* net.

The demand for a new (enlarged) edition of this entertaining and helpful introduction to anatomical study is sufficient proof of its popularity with the student for whom it so paternally caters. Its mingled wit and wisdom afford the beginner a sane orientation and a clear perspective of his subject, whilst, throughout, its seasoned advice and friendly encouragement will assist him in combating his difficulties.

The teacher, too, will profit from this work; he might ponder with advantage the statement (p. 84) relative to students and the taking of trouble.

Whilst endorsing heartily the author's admonitions and suggestions, we must demur from his statement (p. 5) concerning epiphyses in the infantile period, and from that (p. 83) concerning the abolition of systematic lectures.

Magendie's name is misspelt on p. 43, and on p. 77 the brothers Jamieson exchange academic rank. A more unfortunate blemish is the wholly misleading attempt (p. 91) to equate the 50 per cent. pass-mark with "half-knowledge". The purely arbitrary examination standard (whatever its precise percentage) is merely a convenient formula which neither has, nor can have, strict mathematical relationship to the 100 per cent. of "all-knowledge"—itself beyond human acquisition.

Russell A. Hibbs. Pioneer in Orthopaedic Surgery, 1869–1932. By GEORGE M. GOODWIN. (London: Oxford University Press.) 1935. Pp. x+136, Figs. 16. Price 10*s.* net.

This is a fascinating story of a man who, in spite of seemingly insuperable obstacles, won fame as a leading authority and pioneer of orthopaedic surgery in America.

He was brought up on a farm. His father could only provide him with very scanty means when he entered a little-known medical school, where he obtained his qualification in two terms of six months each. Thus equipped with very rudimentary medical knowledge he practised his profession for two years amidst a rural community. However, fired with insatiable ambition, he eventually betook himself to New York where, though at first absolutely unknown, his outstanding personality and indefatigable energy won recognition. An appointment to the Orthopaedic Hospital gave his originality of mind and technical imagination full scope. He devised a new and effective method of lengthening the tendo Achillis for the relief of talipes equino varus, the result of infantile paralysis, and was most successful in his treatment of joints affected with tubercular disease by novel methods of surgical fusion.

The book contains descriptions, illustrated by figures, of his more important operations, and contains a bibliography of his many contributions to surgical literature.

Histochimie Animale, Méthodes et Problèmes. By L. LISON. (Paris: Gauthier-Villars.) 1936. Price 50 fr.

In the opening four chapters of this excellent handbook, the author discusses the problems and general methods of histochemistry. He draws a sharp distinction between true histochemical tests, which should be specific and capable of being explained

in chemical or physical terms, and those tests which are purely tinctorial reactions. Since many histochemical tests rely on the formation of a new compound from the reaction of a substance in a tissue section with a reagent, the limits of microscopic vision have an important bearing on the interpretation of these tests. The author in discussing the well-known prussian blue reaction, which is specific for iron if the latter is present in sufficient concentration to give visible particles of the blue compound, wisely stresses the point that a negative result with this and many other tests does not indicate that the substance is necessarily absent. The average histologist or pathologist who uses histochemical methods quoted in current technical handbooks, must be prepared for a series of dénouements which follow in the chapters dealing with the tests for special organic and inorganic substances. Few of the so-called classical histochemical tests are above suspicion.

The chapter on fats and allied substances is a valuable summary of recent critical methods to which Dr Lison has made several personal contributions. Cytologists, in particular, should give serious consideration to the quotation in chapter 12, from the well-known monograph of Leathes and Raper, concerning the term lipoid ("which is at once a cloak for ignorance and an indefinable limbo in which one can thrust anything of which he knows little or nothing").

An endeavour to improve the cytological fixation of tissues in preparation for histochemical tests, will be noted in many of the new and revised methods for the detection of special substances. The author, for instance, does not hesitate to describe Carnoy's fluid as the worst possible fixative for a tissue in which the presence of glycogen is to be demonstrated. The technique of Giroud and Leblond for the detection of Vitamin C is dealt with in the concluding chapter and appears to be accepted without much reservation, even though it does entail the use of silver nitrate.

A useful bibliography is to be found at the conclusion of each section dealing with the tests for special substances. It will be noted from the lists of references that the subject of histochemistry has not received much attention in this country in recent years. The present handbook should provide a stimulus to rectify this state of affairs.

Handbuch der vergleichenden Anatomie der Wirbeltiere. Edited by the late L. Bolk, E. Göppert, E. Kallius and W. Lubosch. (Berlin: Urban and Schwarzenberg.) 1933. Vol. VI, pp. xii + 854, 629 figs., and 5 coloured plates. Price: single volume, unbound, RM. 96, bound, RM. 102, or part of entire work, RM. 87 and RM. 96.

The first half of vol. VI is devoted to the urogenital system (exclusive of the urinary system, to be dealt with in vol. V) and the second half to the vascular system. The volume opens with an excellent and most useful account of the comparative anatomy of the gonads and their efferent ducts by Prof. van der Brock of Utrecht. The genital organs of the Monotremes and Marsupials are dealt with at some length, and there is an interesting discussion of the problem of testicular descent incorporating the author's own views. The illustrations, many of them original, are deserving of special commendation. In Chapter II, Dr de Lange, Director of the Hubrecht Laboratory, Utrecht, appropriately enough provides a most useful review of the Mammalian placenta. After a brief introduction, he first summarises our knowledge of the placentation of the Marsupials, on the basis of the work of Hill and Flynnand and then, following the classification of Grosser he gives an account, in the reference to particular examples, of the four types of placenta recognised by that authority. He includes descriptions of the placentae of little-known forms like Manis, Orycteropus, Tupaia, Chrysochloris, Ealeipithecus, largely based on original observations on the

rare material contained in the Hubrecht Laboratory. Chapters III and IV, by Prof. H. Stieve and Prof. J. Hett respectively, are quite short and deal with the interstitial cells of the gonads and the comparative anatomy of the corpus luteum. Next follows a very informative chapter (V) by that well-known authority, Prof. N. Gerhardt, on the cloaca and copulatory organs, a mine of information in all that relates to the comparative morphology of these latter structures in the vertebrate series. The account of the urogenital system is concluded by a short but interesting description by Prof. H. v. Eggeling of the musculature of the pelvic outlet in Mammals. After a brief review of the essential facts in lower vertebrates, he describes the muscles originating from the pelvis and inserted into the tail, the sphincter cloacae and its derivatives and the smooth muscle of the rectum and urogenital canal.

The second half of the volume opens with a suggestive chapter by Prof. F. Weidenreich on the general morphology of the vascular system. Specially valuable is the detailed comparative account of blood cells in the vertebrate classes. In discussing the theories of their formation, he supports the monophyletic school. Then follows an account, largely histological, of the organs of circulation, regulation and distribution. The final section (25 pp.) is devoted to the "hämolymphatische Gewebe", as the author terms it in consonance with the conception that the vascular and lymphatic systems evolved from a common haemolymph system. A detailed account is given of the histology of the spleen, lymphatic and haemolymph glands.

Chapter II, by Prof. F. Franz, is devoted to a detailed description of the vascular system of that basal Chordate type, Branchiostoma (Amphioxus), the author supplementing and correcting his earlier descriptions through new observations.

In Chapter III, Prof. A. Benninghoff provides a summary of existing knowledge of the comparative anatomy of the vertebrate heart. His account of the reptilian heart is largely based on the work of Greil and but scanty reference is made to that of British investigators. Of special value is the author's account of the architecture of the heart muscle in which he summarises his own researches. The chapter concludes with a useful comparative account of the conducting system, in which the fundamental observations of Keith and Flack and Mackenzie are duly recorded, but no mention is made of the work of Lewis (1917). In Chapter IV (3 pp.) Prof. Tandler provides a sketch of the branchial circulation and its transformation. Chapter V (pp. 563–684) by Prof. A. Hafferl is devoted to a systematic description of the arterial system in the vertebrate classes, a monumental summary of the greatest value for reference purposes, accompanied as it is by a very full bibliography. The description of the venous system is undertaken by Dr C. van Gelderen in Chapter VI. The author gives a detailed description of the venous system in the various vertebrate groups and in a final section an account of the vitelline and placental circulations in the embryo and foetus.

The seventh and last chapter opens with an illuminating discussion of the lymphatic system, its relations and origin, by Prof. F. Weidenreich, who is also responsible for the detailed account of the system in Fishes, Amphibia and Reptilia, whilst the late Prof. Baum and Prof. Trautmann undertake its description in the Birds and Mammals. This is one of the most valuable chapters in the volume, since it provides for the first time a comparative survey of the lymphatic system in the vertebrates as a whole.

This volume is a definite addition to the literature of Comparative Anatomy and we commend it to the attention of all anatomists. It is well printed and contains many original illustrations, extremely well reproduced. Bibliographic lists are appended to the chapters, and though not perfect should prove extremely useful to research workers. There is no index, but a full list of contents is provided.

Handbuch der vergleichenden Anatomie der Wirbeltiere. Herausgegeben von L. Bolk, E. Göppert, E. Kallius und W. Lubosch. Band II. (Berlin: Urban und Schwarzenberg.) 1934. Pt. I, x + 1–840, Figs. 1–610; Pt. II, vii + 841–1444, Figs. 611–1279. Price: single volume, RM. 165; or part of entire work, RM. 150.

This volume is divided into two parts—Part I being devoted to the cerebrospinal and sympathetic nervous system, and Part II to the sense organs. Von Haller von Hallerstein has written the sections on the gross structure of the brain and spinal cord and on the cranial nerves. The former section is arranged so that each of the great divisions of the central nervous system is considered separately—a method which allows of easy reference but which has the disadvantage of making difficult the understanding of the central nervous system as a whole in the various vertebrate groups. This disadvantage is lessened by the masterly treatment of the section on the finer structure of the brain and cord by Ariens Kappers, who has been able to compress into less than 200 pages not only an excellent description of vertebrate brain structure, but a critical treatment of the problems of brain morphology based, to a great extent, not only on his own extensive work but on that of most modern investigators. The brief but delightfully written section by Boeke on the histogenesis of the peripheral nerves contains an excellent description of the early development of nerves and their connections with the myotomes and epithelium which takes into account most of the recent work in this field. Van der Horst is responsible for the section on the limb plexuses. The cranial nerves are described by von Haller von Hallerstein. He has devoted considerable space to a consideration of the lower vertebrates and especially to the vagal complex of these animals. Hirt's description of the gross anatomy of the sympathetic nervous system is based largely on the work of Vierling, van den Brock and on his own investigations. This section will be of considerable value to those who are undertaking experimental work on the peripheral sympathetic system. Van der Sprenkel in a short article summarises our knowledge of the sympathetic paraganglia and the supra-renal body.

The second part of this volume deals with the sense organs. Boeke writes the two opening sections—the first on invertebrate sense organs and the ontogeny and distribution of sense organs, the second on free nerve endings and the endings of sensory nerves. A third article by him on the less known end-organs in the lower vertebrates is a useful summary. The chapter on the olfactory organ and the accessory organs of the nose is written by Matthes but is condensed too much to be of value, although the major facts are well presented. Some expansion at the expense of the following article by Franz on the eye would have been justified. The chapters on the eye are extremely detailed, but the section devoted to the structure of the retina of the mammals leaves much to be desired. De Burlett has produced excellent chapters on the structure of the internal and middle ear. The author is to be congratulated on the beautiful preparations with which he has illustrated his article. A short chapter on the external ear by Boas completes the volume.

The bibliographies are exhaustive and illustrations are numerous, but the absence of an index is a serious omission.

It is proposed that Volume LXXI, 1936–37 of the *Journal of Anatomy* be dedicated to Sir Grafton Elliot Smith, F.R.S., because of his many valuable contributions to biological science.

THE THALAMIC CONNECTIONS OF THE TEMPORAL LOBE OF THE BRAIN IN THE MONKEY

By W. E. LE GROS CLARK

Department of Anatomy, University of Oxford

One of the most accurate methods of determining the precise relation between the different nuclear elements of the thalamus and the cerebral cortex is the study of secondary cell atrophy in the thalamus following local cortical ablations. This method was used in a survey of the parietal and frontal lobes of the monkey (Clark and Boggon, 1935), and it was there shown that in all cases where atrophy ensued in the thalamus it was clear-cut and sharply localised. It was thus comparatively simple to detect the cell groups which were related by thalamo-cortical fibres to the limited cortical areas involved by the lesions. The present communication represents an extension of this study of the macaque brain.

METHODS

The observations here recorded are based on five experiments. Ordinary macaque monkeys (*Macaca mulatta*) were used for the purpose. In each, a small block of cortex was removed from the temporal lobe under nembutal anaesthesia. As the records of the experiments show, the lesions were relatively superficial, being practically limited to the cortex and subjacent white matter. The animals made rapid recoveries from the operations and showed no observable functional defect as a result of the cortical injury. They were allowed to survive for two to three months, after which they were killed by an overdose of anaesthetic. On death the brain was immediately removed and studied macroscopically. The lesion was measured and as accurately as possible reproduced diagrammatically. It should be noted that these diagrams of the cortical lesions (reproduced below) make the lesions appear rather less extensive than they actually were at the time of the operation, owing to the subsequent contraction of the tissues during the process of healing. In each case the area of cortex containing the lesion was embedded in paraffin, sectioned, and stained with methylene blue. In this way the extent of the damage to the cortex could be ascertained, with special reference to the different cortical areas which had been involved (see Plate I, fig. 1). As soon as the cortical lesion had been studied macroscopically, a block was removed from the brain containing the whole extent of the thalamus. This was placed in 70 per cent. alcohol for one or two days until sufficiently hardened, when it was cut into separate blocks 2–3 mm. in thickness which were embedded in paraffin. Complete serial sections were cut throughout the length of the thalamus in a coronal plane at 15 μ, and were stained with Borell's methylene blue.

The experiments showed that the temporal lobe receives thalamo-cortical fibres from the medial geniculate body and the pulvinar. In order to make the description of the results clear, a short account of the constitution of these thalamic elements in the normal brain is given here.

The medial geniculate body (Plate I, fig. 2A). It has now been definitely established that the medial geniculate body is a thalamic element which developmentally is derived from the same mass of cells which also gives rise to the ventral nucleus of the thalamus (Clark, 1933). In the macaque monkey it measures approximately 3 mm. in antero-posterior diameter, 3·5 mm. vertically, and 2 mm. transversely. Friedemann (1911–12) in *Cercopithecus* recognises a caudo-ventral small-celled element, an oro-dorsal element in which the cells are of medium size, a well-defined lateral element, and the nucleus parageniculatus (*noyau basal C* of Vogt). In *Macaca* there seems to be no clear differentiation between oro-dorsal and caudo-ventral elements, though the caudo-ventral region of the main part of the medial geniculate body does contain a greater proportion of small cells. The pars lateralis forms a very distinct element containing relatively large and closely packed cells, and occupies the dorso-lateral angle of the geniculate body. It is marked off from the main part of the latter by a thin medullary lamina, and it can be followed to its anterior extremity. It is not distinguishable at the caudal pole of the geniculate body. The parageniculate nucleus consists of somewhat scattered, large, and deeply staining cells which caudally appear at the ventro-medial angle of the medial geniculate body at about the middle of its length. Traced forwards, these cells extend up along the medial border of the main part of the geniculate body in a dorso-medial direction and appear here to be a ventro-lateral extension of the nucleus limitans. As will be seen, the evidence indicates that the parageniculate nucleus is not connected to the auditory cortex (or, indeed, to any part of the cortex), and it probably should not be included as an element of the medial geniculate body.

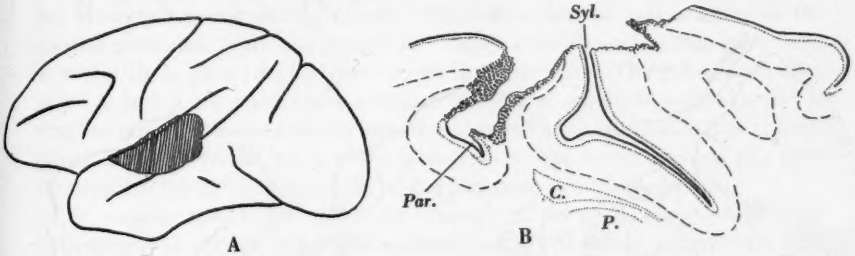
The pulvinar. Nissl sections through the posterior projecting pole of the pulvinar in the macaque brain show that it here contains evenly distributed nerve cells of medium size in a comparatively clear matrix. This is the caudal extension of the main nuclear element of the pulvinar, nucleus $p\beta$ of Friedemann. Farther forwards, where the pulvinar becomes continuous laterally with the internal capsule, the cells in its lateral part are rather larger and tend to be arranged in rows among the fasciculi of the thalamo-cortical radiations. This slight cyto-architectural differentiation corresponds to Friedemann's nucleus $p\mu$. At the level of the caudal end of the medial geniculate body, the cells of the ventral margin of nucleus collect to form a well-defined group occupying the ventral projecting angle of the pulvinar. This group extends forwards without interruption to become continuous with the nucleus intergeniculatus (nucleus *pd* of Friedemann). The intergeniculate nucleus is quite a sharply circumscribed element in rostral planes, and forms a triangular mass of cells interposed between the medial and lateral geniculate bodies. It is

bounded dorsally by cortico-tectal fasciculi which serve here to mark it off from the main element of the pulvinar, $p\beta$. Where the cortico-tectal fasciculi form compact groups of fibres, the cells of the pulvinar round them become somewhat condensed, and this local differentiation comprises the element $p\gamma$ of Friedemann. At the dorso-medial angle of the pulvinar (which overhangs the lateral margin of the anterior colliculus), a further slight differentiation in which the cells are rather smaller and more scattered forms the nucleus $p\alpha$. This element is also defined by a medullary lamina which circumscribes it laterally. Lastly, in the rostral part of the pulvinar, elements $p\beta$ and $p\mu$ blend to form a group of cells termed nucleus $p\mu\beta$ by Friedemann.

EXPERIMENTAL RESULTS

Exp. A. Monkey O.M. 103. Operated 11 October 1935, killed 7 December 1935.

A lesion was made in the superior temporal gyrus about midway along its length. Measured after death, the lesion was found to be 22 mm. long and 7·5 mm. wide. It reaches forward to 10 mm. from the Sylvian point, while its



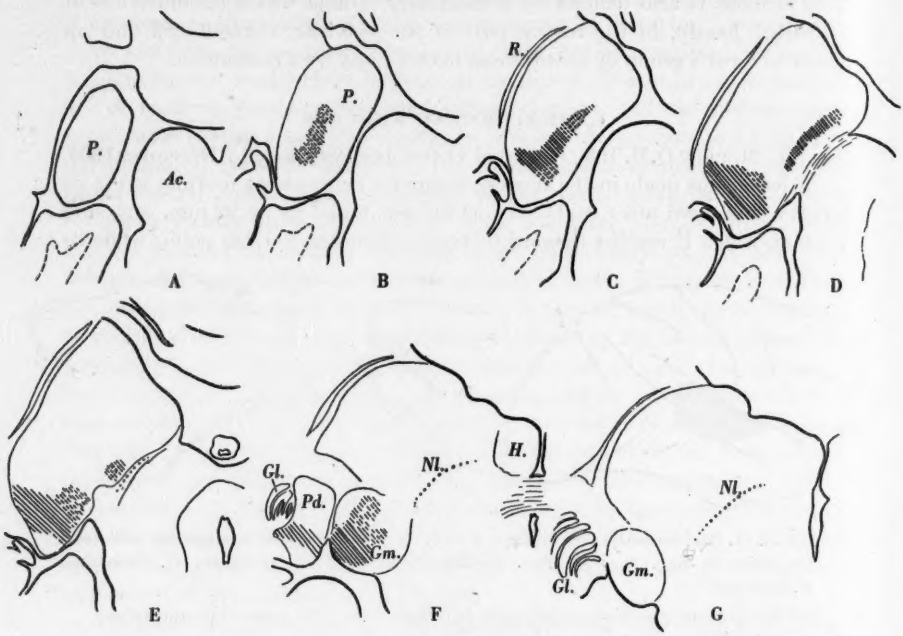
Text-fig. 1. A. The cortical lesion in Exp. A. ×⅔. B. Vertical section through the middle of the lesion in Exp. A. ×3⅓. *Par.* Parallel sulcus. *Syl.* Sylvian sulcus. *C.* Claustrum. *P.* Putamen.

(All the diagrams of sections in this paper have been made with a projection apparatus.)

posterior margin is 4 mm. from the anterior extremity of the inferior lunate sulcus. Above and behind it extends up to 8 mm. from the caudal extremity of the Sylvian sulcus. Below it involves the parallel sulcus to a slight extent (text-fig. 1 A). Nissl sections cut across the middle of the lesion show that it involves almost the whole width of the superior temporal gyrus at this level, destroying much of the submerged upper surface of the gyrus and interrupting a proportion (but by no means all) of the fibre connections of the portion of this cortical area which remains. The lesion also extends slightly into the upper margin of the middle temporal gyrus, and the dorsal lip of the Sylvian sulcus (text-fig. 1 B).

The thalamus (text-fig. 2). Tracing the serial sections forwards, the free pole of the pulvinar first appears in section 8 of the first block. There is no sign of cell degeneration at the tip of the pulvinar. In section 92, at the level where the pulvinar comes into continuity with the internal capsule laterally,

a definite zone of cell atrophy with an accompanying gliosis appears in the centre of the pulvinar (text-fig. 2 B). This zone involves the element $p\beta$ of Friedemann. At first scattered normal cells are to be seen in the area of degeneration, but a few sections farther rostrally these mostly disappear, the zone of atrophy is much more sharply defined and extends to the ventral surface of the pulvinar, while the gliosis is more dense. At the level where the pulvinar becomes adherent to the side of the mid-brain (section 136), the



Text-fig. 2. Transverse sections through the caudal part of the thalamus in Exp. A. Areas of complete or practically complete cell atrophy are indicated by hatching with continuous lines, areas showing partial cell atrophy by interrupted lines. *Ac.* Anterior colliculus. *Gl.* Lateral geniculate body. *Gm.* Medial geniculate body. *H.* Habenular ganglion. *Nl.* Nucleus limitans. *P.* Main element of pulvinar. *Pd.* Intergeniculate nucleus. *R.* Nucleus reticularis.

area of atrophy is somewhat larger, extending here through the ventral and medial part of the pulvinar, but the group of cells which occupies the ventral projecting angle of the pulvinar has escaped degeneration (Plate II, fig. 1). A few sections forwards, the zone of atrophy becomes separated into two areas. Of these, the dorsal is relatively small and not so sharply circumscribed. It extends along the lateral aspect of the cortico-tectal fasciculi, involving here the element $p\gamma$ of Friedemann. The ventral area occupies most of the ventral angle of the pulvinar, being very well defined, and it involves the ventral part

of elements $p\beta$ and $p\mu$ (fig. 2 D). The dorsal area of atrophy reaches forwards for a short distance, lying alongside the dorso-lateral aspect of the nucleus limitans and finally disappears in section 212. At this level the ventral area occupies the ventral extremity of $p\mu$ and the caudal end of the nucleus intergeniculatus (*pd*).

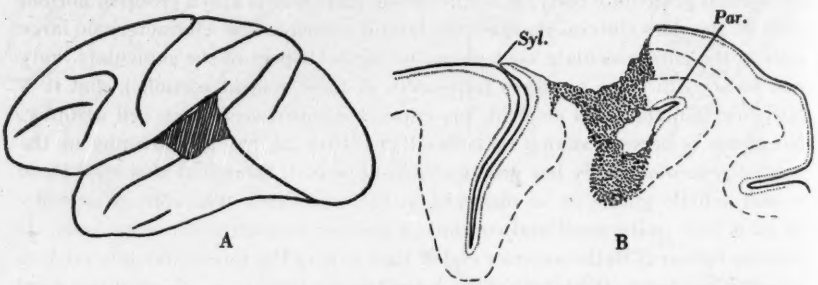
Section 1 of the next block cuts through the caudal part of the medial geniculate body, the caudal extremity of the lateral geniculate body, and the intergeniculate nucleus. Here there is still a well-defined though small patch of cell atrophy in element *pd*, close to the hilum of the lateral geniculate body. Most of the medial geniculate body at this level is also involved in the cellular degeneration (text-fig. 2 F). A zone of small cells at the ventral margin (evidently corresponding to a part of Friedemann's caudo-ventral element of the medial geniculate body) is not involved, and there is also a group of normal cells at the dorso-lateral margin, the lateral element. The characteristic large cells of the parageniculate nucleus on the medial aspect of the geniculate body can be seen in large numbers (especially in more rostral sections), and it is fairly certain that this element has entirely escaped secondary cell atrophy. No gliosis is present among its cells. By section 22, the cell atrophy in the medial geniculate body has practically disappeared, though at this level there is still a little gliosis to be observed in the same area. The zone of atrophy in *pd* is here quite small and contains a number of scattered normal cells. It reaches forwards to the anterior end of that part of the intergeniculate nucleus which is related to the hilum of the lateral geniculate body. Beyond this level (section 40) the cyto-architecture of the thalamus is entirely normal.

It may be noted that the rostral margin of the medial geniculate body corresponds to section 136 of the second block. Thus, in the anterior 1·7 mm. of the nucleus the cells are quite normal, the cell atrophy resulting from the lesion in the temporal lobe being confined to the caudal half. The zone of atrophy in the pulvinar extends back to a level 1·25 mm. from the free tip of this part of the thalamus, and extends forwards as far as the rostral extremity of the intergeniculate nucleus.

Exp. B. Monkey O.M. 94. Operated 3 October 1935, killed 4 December 1935.

A lesion was made in the superior temporal gyrus. On examination of the brain at death, it was found to measure 8 mm. in vertical diameter and 15 mm. antero-posteriorly. The anterior extremity reaches to a point just below the inferior extremity of the central sulcus, while the posterior extremity is 3 mm. from the posterior end of the Sylvian sulcus. Below, the lesion cuts across the parallel sulcus, and just involves the anterior end of the inferior lunate sulcus (text-fig. 3 A). Transverse sections through the middle of the lesion (Plate I, fig. 1 and text-fig. 3 B) show that it is not so deep as in Exp. A, and involves only a small extent of the submerged upper surface of the superior temporal gyrus. It is confined to Brodmann's area 22, the upper border of area 21, and a part of areas 18 and 19.

The thalamus. Tracing the serial sections forwards, it is noted that at the free tip of the pulvinar the cell architecture and density are quite normal. Counting from the section at which the pulvinar is first seen, in section 105 a zone of cell rarefaction with accompanying gliosis appears in the centre of the pulvinar at the level where the latter begins to run into continuity with the side of the mid-brain. This zone becomes gradually better defined and the gliosis more dense as the sections are traced rostrally. In section 130 there is a clearly circumscribed area of total cell atrophy occupying the medial and ventral part of the pulvinar, reaching towards its ventral angle and along the dorso-lateral aspect of the cortico-tectal fasciculi. It involves portions of elements $p\beta$ and $p\gamma$ (text-fig. 4 B). A few sections farther forwards the area enlarges somewhat and soon becomes divided into two separate zones (section

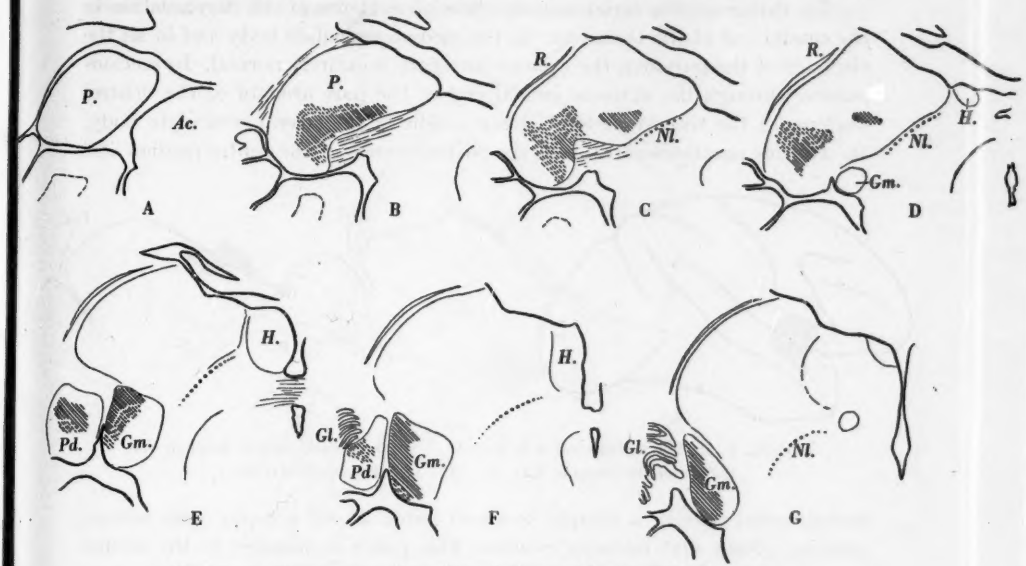


Text-fig. 3. A. The cortical lesion in Exp. B. $\times\frac{2}{3}$. B. Vertical section through the middle of the lesion in Exp. B. $\times 3\frac{1}{3}$.

160, text-fig. 4 C). The dorsal zone is small but very sharply defined, and lies in the element $p\beta$ lateral to the middle of the nucleus limitans. The ventral zone is more diffuse and larger. It extends towards the ventral margin of the pulvinar, and the greater part of it contains a fair number of scattered normal cells in the midst of a well-marked gliosis. It also involves element $p\beta$ of the pulvinar. In section 170, the caudal extremity of the medial geniculate body appears, as well as the caudal end of the intergeniculate nucleus (element *pd*). Both these structures are normal at this transverse level (text-fig. 4 D). On the other hand, the ventral area of cell atrophy in element $p\beta$ is here very distinct, while the small dorsal area is undergoing rapid reduction.

The first section of the next block passes through the central part of the medial geniculate body and the intergeniculate nucleus, but still lies behind the level of the lateral geniculate body. In this section there is a very distinct zone of cell atrophy and gliosis in the dorso-lateral part of the intergeniculate nucleus and also in the dorso-lateral angle of the medial geniculate body (text-fig. 4 E). The degeneration in the medial geniculate body rapidly extends and, at the level of the caudal end of the lateral geniculate body, it involves the whole of its dorsal half. The parageniculate nucleus is quite intact. The area of atrophy in the intergeniculate nucleus, on the other hand, becomes

smaller in more rostral sections, and less well defined. By section 45 this area has practically disappeared except for a slight gliosis in the same region. In this section the zone of atrophy in the medial geniculate body occupies its lateral half, except for a zone of small cells at its ventral border. These cells appear normal (text-fig. 4 F and Plate I, fig. 2*b*). At the level of sections passing through the middle of the lateral geniculate body, the atrophy involving the rostral pole of the medial geniculate body is extremely well defined



Text-fig. 4. Transverse sections through the caudal part of the thalamus in Exp. B. Lettering, etc., as in text-fig. 2.

and sharply demarcated from the normal medial half (section 86, text-fig. 4 G). It extends in this position forwards to the rostral extremity of the medial geniculate body (section 145).

In this experiment the extent of the cell atrophy in the projecting pole of the pulvinar is very similar to that of the previous experiment. It reaches back to within 1·6 mm. of the free tip. On the other hand, the caudal ends of the intergeniculate nucleus and the medial geniculate body have escaped degeneration. Approximately the dorso-lateral half of the medial geniculate body has undergone complete cell atrophy for at least its anterior 2 mm.

Exp. C. Monkey O.M. 88. Operated 8 September 1935, killed 2 December 1935.

The lesion in this case was made in the superior temporal gyrus. At death it was found to be fairly small, measuring superficially 13 mm. in vertical diameter and 8 mm. antero-posteriorly. It involves the superficial and upper surfaces of the superior temporal gyrus, reaching deeply into the Sylvian

fissure and into the insular cortex (text-fig. 5). At one point, the lesion just touches the claustrum. Above, the extreme lower end of the motor cortex is involved, and in the sections Betz cells can be recognised immediately adjacent to the margin of the lesion. The anterior border of the lesion is 6·5 mm. from the Sylvian point, the lower border extends down to 2·5 mm. from the parallel sulcus, the posterior border is 2 mm. from the lower end of the central sulcus, and the antero-superior angle lies 3 mm. distance from the arcuate sulcus.

The thalamus. The serial sections show no evidence of cell degeneration in the caudal end of the thalamus. In the medial geniculate body and in all the elements of the pulvinar, the cyto-architecture is entirely normal. In sections passing through the extreme caudal end of the pars arcuata of the ventral nucleus (at the transverse level of the middle of the lateral geniculate body, the nucleus parafascicularis, and the posterior ends of the centre median and

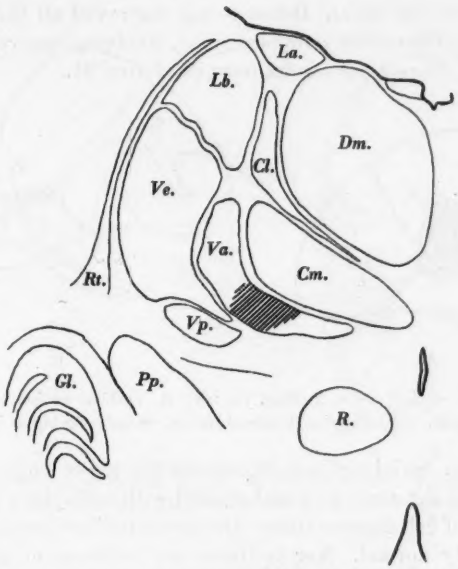
Text-fig. 5. A. The cortical lesion in Exp. C. ×⅔. B. Vertical section through the middle of the lesion in Exp. C. ×3¼. Lettering as in text-fig. 1.

dorso-medial nuclei), a sharply localised patch of cell atrophy with accompanying gliosis first becomes evident. This patch is situated in the medio-ventral end of the arcuate nucleus. Farther forwards the atrophic area becomes rapidly more conspicuous and circumscribed. At the transverse level of its maximum development it measures 1·3 mm. in transverse diameter and 1·0 mm. vertically. Its position at this level is shown in text-fig. 6 and Plate II, fig. 2. From these illustrations it can be seen that the atrophy is confined to the lateral part of the medio-ventral half of the arcuate nucleus, the medio-ventral quarter of this element being normal. The microphotograph in Plate II shows the practically complete absence of normal cells in the patch of atrophy, the conspicuous gliosis, and its sharp contour. It extends through the thickness of the arcuate nucleus and is limited abruptly above by the intermediate medullary lamina which separates it from the centre median nucleus. There is no cell degeneration in the pars externa or the pars posterior of the ventral nucleus.

The atrophic area in the arcuate nucleus can be traced through the whole thickness of the second block of sections (104 sections = 1·6 mm.). The first section of the third block passes through the rostral end of the centre median nucleus and the arcuate nucleus. In the latter some gliosis and slight cell

atrophy are still to be noted in its medio-ventral half. But in 10 sections or so the rostral extremity of the arcuate nucleus is reached and the patch of cell degeneration also disappears. There is no cell atrophy in the other thalamic elements (i.e. lateral, anterior, centre median, dorso-medial or intralaminar nuclei).

The sharply localised patch of cell atrophy in the pars arcuata of the ventral nucleus is very striking in this case. It is undoubtedly related to the involvement by the cortical lesion of the lower end of the motor cortex

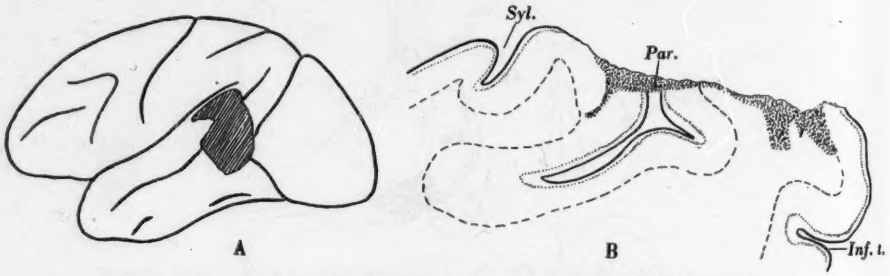


Text-fig. 6. Transverse section through the middle of the thalamus in Exp. C, showing the extent of cell degeneration in the arcuate nucleus. *Cl.* Nucleus centralis lateralis. *Cm.* Centre median nucleus. *Dm.* Dorso-medial nucleus. *Gl.* Lateral geniculate body. *La.*, *Lb.* Elements of lateral nucleus. *Pp.* Pes pedunculi. *R.* Red nucleus. *Rt.* Nucleus reticularis. *Va.* Ventral nucleus, pars arcuata. *Ve.* Ventral nucleus, pars externa. *Vp.* Ventral nucleus, pars posterior.

(area 4). Previous experimental work (Clark and Boggon, 1935) has provided evidence that the arcuate nucleus projects on to the motor cortex. This conclusion was largely based upon an experiment in which the upper half of the precentral gyrus was destroyed, and the resulting atrophy in the arcuate nucleus was most conspicuous in its dorso-lateral part. In the experiment recorded here, the atrophy in the ventro-medial part of the nucleus following a lesion in the lower end of the precentral gyrus adds corroboration to the inference that the whole of the arcuate nucleus projects on to the motor cortex.

Exp. D. Monkey O.M. 87. Operated 25 September 1935, killed 27 November 1935.

The lesion was made in the posterior part of the temporal lobe. At death it was found to measure 16 mm. in vertical diameter, and 14 mm. antero-posteriorly. The anterior margin is 23 mm. behind the Sylvian point. The lesion involves the superior temporal gyrus as far back as the rostral margin of the area striata, just reaching here to the anterior extremities of the superior and inferior lunate sulci (text-fig. 7). Coronal sections taken through the middle of the lesion show that the injury to the superior temporal gyrus (area 22) is quite superficial, Below it has destroyed all the exposed part of the caudal end of the middle temporal gyrus, involving here the middle portion of areas 18 and 19 and the caudal margin of area 21.



Text-fig. 7. A. The cortical lesion in Exp. D. $\times\frac{2}{3}$. B. Vertical section through the lesion in Exp. D. $\times 3\frac{1}{4}$. *Inf.t.* Inferior temporal sulcus. Other lettering as in text-fig. 1.

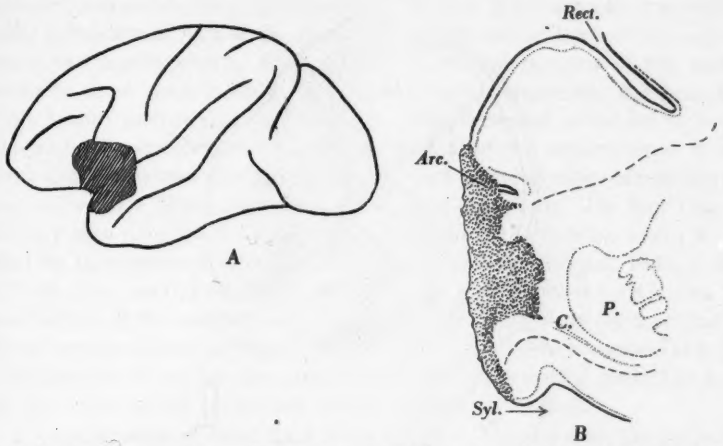
The thalamus. Serial sections throughout the whole length of the thalamus were prepared in the usual way and stained with methylene blue. Nowhere is there any sign of cell degeneration—the cyto-architecture of all the thalamic nuclei is entirely normal. Nor is there any evidence of gliosis among the thalamic radiations as is found in cases in which cell degeneration in the thalamus follows cortical lesions. It is to be concluded, therefore, that the area of cortex destroyed by the lesion in this experiment does not receive any thalamo-cortical fibres.

Exp. E. Monkey O.M. 102. Operated 8 October 1935, killed 25 November 1935.

The lesion was made at the temporal pole. Examination of the brain at death showed that it extends into the orbital operculum. It measures 16 mm. in vertical diameter and 14 mm. antero-posteriorly. Below it reaches to within 1 mm. of the anterior extremity of the parallel sulcus while above it involves the lower end of the arcuate sulcus (text-fig. 8 A). Vertical sections through the lesion showed that it involves the superficial aspect and upper surface of the cortex of the temporal pole (area 22), while comparison with Brodmann's and Mauss's charts of the cercopitheque brain makes it certain that above it extends into the lower margin of areas 4 and 6 and into the caudo-ventral

angle of areas 8 and 9 (text-fig. 8 B). The cortex of the insula is not involved, and the basal ganglia are likewise intact.

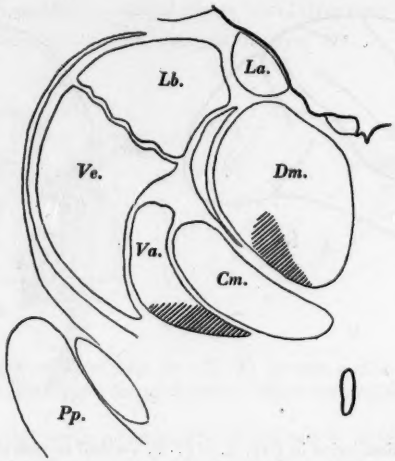
The thalamus. The serial sections through the first block show that the cyto-architecture in the caudal end of the thalamus is entirely normal. There is no sign of cell degeneration or gliosis in the medial geniculate body or in any of the elements of the pulvinar. The first section of the second block cuts through the caudal extremity of the dorso-medial nucleus, and at this level, also, all the nuclear elements of the thalamus are normal. The caudal end of the arcuate nucleus first appears in section 15. In section 60 a small but



Text-fig. 8. A. The cortical lesion in Exp. E. $\times \frac{2}{3}$. B. Vertical section through the lesion in the orbital operculum in Exp. E. $\times 3\frac{1}{3}$. *Arc.* Arcuate sulcus. *Rect.* Sulcus rectus. Other lettering as in previous figures.

distinct patch of complete cell atrophy and gliosis is evident in the extreme medio-ventral corner of this nucleus, immediately ventral to the inner end of the centre median nucleus. At the same transverse level a slight gliosis is to be observed at the medio-ventral angle of the small-celled portion of the dorso-medial nucleus. A few sections farther rostrally, the area of gliosis in the dorso-medial nucleus is replaced by a well-defined patch of cell atrophy and gliosis. These degeneration areas in the dorso-medial and arcuate nuclei become rapidly more distinct. The former is sharply defined ventrally by the medullary lamina separating the dorso-medial from the centre median and the parafascicular nuclei. The atrophic area in the arcuate nucleus is similarly circumscribed by the medullary lamina around its medial end (Plate II, fig. 3). This latter area remains stationary through many sections as it is traced forwards. At section 105 it extends slightly laterally along the ventral border of the arcuate nucleus but always retains its clear-cut outline. It reaches its greatest transverse development at about section 150, where it

measures 2·0 mm. in transverse width and 0·7 mm. in vertical width (text-fig. 9). Thereafter, the patch diminishes in size until by section 180 it finally disappears. The patch of atrophy in the dorso-medial nucleus also reaches its maximum development at about section 150 where it measures 2 by 1·5 mm. Farther forwards there appears some slight diffuse gliosis in the lateral half of the dorso-medial nucleus, but this is not accompanied by any definite evidence of cell degeneration. The atrophic area at the medio-ventral angle of the nucleus begins to disappear at the rostral end of the second block, that is to say, towards the anterior end of the nucleus. There is no cell degeneration



Text-fig. 9. Transverse section through the middle of the thalamus in Exp. E, showing the extent of the cell degeneration in the arcuate and dorso-medial nuclei. Lettering as in text-fig. 6.

or gliosis to be observed in any other thalamic elements. The cyto-architecture of the pars externa of the ventral nucleus, the lateral nucleus, centre median nucleus, anterior nucleus and the intralaminar nuclei is quite normal.

In this experiment the cortical lesion had involved, besides the temporal pole, the ventral borders of the motor cortex (area 4) and of the area frontalis granularis. Previous experiments (Clark and Boggon, 1935) have shown that these areas are related by thalamo-cortical fibres with the pars arcuata of the ventral nucleus and the dorso-medial nucleus respectively. These conclusions are confirmed in a striking manner in the present instance. For small circumscribed patches of cell atrophy are found sharply localised at the medio-ventral extremity of the arcuate nucleus, and at the medio-ventral angle of the dorso-medial nucleus. On the other hand, the destruction of the temporal pole, involving the anterior end of cortical area 22, is not reflected in any evident cell atrophy in the thalamus.

DISCUSSION

The results of the experiments recorded in this communication may most conveniently be discussed by considering separately the thalamic nuclei affected.

Medial geniculate body. In two experiments, A and B, secondary cell degeneration in this nucleus was found. In both these cases the cortical lesion had involved the superior temporal gyrus immediately below the lower end of the central sulcus. In Exp. A the lesion extended somewhat deeper into the submerged dorsal surface of the gyrus, and in this case the cell atrophy extended deeper into the geniculate body. In Exp. B the atrophy was confined to the dorso-lateral part of the nucleus. That the rostral limit of the auditory cortex can hardly extend farther than the rostral margins of the cortical lesions in these cases is shown by Exps. C and E (especially the former) in which lesions farther forwards in the superior temporal gyrus led to no cell changes in the medial geniculate body. Exp. D of the present series at least shows that the caudal third of the exposed part of the superior temporal gyrus does not receive fibres from the medial geniculate body. The fact that the auditory area does not extend very far back along the Sylvian sulcus is indicated by an experiment recorded elsewhere (Clark and Boggon, 1935, p. 336), in which the posterior extremity of this sulcus was involved by a lesion. The small extent of the auditory cortex is further emphasised by the fact that the limited cortical lesion in Exps. A and B not only involve a considerable part of this area (as shown by the extensive atrophy in the medial geniculate body) but also a part of the projection of the pulvinar (*vide infra*).

A consideration of these data leads to the conclusion that the projection area of the medial geniculate body is limited to a small area of cortex, probably not more than 15 mm. in length, in the superior temporal gyrus situated immediately below the inferior end of the central sulcus. This conclusion is in complete accord with Poliak's statement (1932) (based on Marchi studies) of the extent of the auditory cortex in the monkey. This author further records that the overwhelming majority of auditory fibres from the medial geniculate body terminate in the submerged cortex on the dorsal surface of the superior temporal gyrus. Thus the audito-sensory cortex is confined to a very small proportion of Brodmann's cortical area 22.

The extent of the cell degeneration in Exps. A and B show that, with the exception of the parageniculate nucleus, all the elements of the medial geniculate body (oro-dorsal part, caudo-ventral part, and pars lateralis) project on to the auditory cortex. In both cases the large cells of the parageniculate nucleus remained unaffected, and it may be surmised, therefore, that they are unconnected with the cortex of the superior temporal gyrus. Walker (1935) has described the cell degeneration in the thalamus of the monkey in two cases of hemi-decortication. In both these cases the parageniculate nucleus (as he has informed me in a private communication) was apparently normal,

whereas the whole of the medial geniculate body proper had undergone complete atrophy. It has already been noted that in the normal thalamus the parageniculate nucleus appears in section to be a caudo-lateral extension of the nucleus limitans. If this is so it is to be regarded not as a part of the medial geniculate body proper, but as an intralaminar nucleus, and Walker has demonstrated that the intralaminar nuclei of the thalamus as a whole do not project on to the cortex.

It is not possible from these experiments to advance detailed evidence on the question as to whether there is any precise localisation within the auditory cortex, that is, whether different sectors of the medial geniculate body project on to corresponding different areas of the auditory cortex. This is a difficult problem to settle, for the auditory cortex is so small in extent and it possesses no well-defined boundaries (such as the lunate and calcarine sulci in the visual cortex) in topographical relation to which small localised lesions might be made and by reference to which the precise position of these lesions could be localised accurately afterwards. Moreover, so far is is known, the auditory cortex in the monkey (unlike the anthropomorphous apes) shows no characteristic structure by which it can be differentiated histologically from the surrounding cortex of area 22 (Mauss, 1911). The very fact that partial lesions of the auditory cortex result in local and sharply circumscribed areas of atrophy in the medial geniculate body (rather than a diffuse partial atrophy affecting the whole nucleus) makes it practically certain that within this nucleus there is a localisation in regard to different sections of the auditory cortex as precise as is known to be the case in the lateral geniculate body with regard to the visual cortex. In this connection it may be noted that Poliak (1932) has remarked on the great regularity of arrangement and distribution of the fibres of the auditory radiation. Each of the bundles composing the radiation preserves its relative position with respect to its neighbour during its entire course up to its cortical termination. Poliak also describes a rotation of the auditory radiation in Primates of such a kind that the ventral bundles of the radiation (emanating from the ventral part of the medial geniculate body) are distributed to the more rostral part of the auditory cortex, while the dorsal bundles terminate in the more caudal part. This he associates with a displacement of the auditory cortex from its primitive vertical position (as seen, for instance, in the Sylvian gyrus in the cat) to the horizontal position which it assumes in the higher Primates with the greater development of the temporal lobe. Poliak concludes that the only interpretation of this rotation of the auditory radiation "must be the necessary preservation of the original mutual relations of the individual bundles and fibres of the radiation to one another and above all to special segments of the internal geniculate body and the auditory cortex. And this in turn renders possible an exact projection of the receptor surface of the auditory peripheral organ, the cochlea, upon the auditory projection cortex." It may be noted that the results of Exps. A and B do suggest a broad scheme of localisation wherein the ventral and dorsal parts of the medial

geniculate body are projected on to the rostral and caudal parts respectively of the auditory cortex. In Exp. A the lesion extends farther forward and the dorsal half of the geniculate body has partly escaped secondary degeneration: in Exp. B, in which the lesion is more caudally situated, the degeneration in the geniculate body is predominantly in the dorso-lateral region.

As regards lower Mammals, a general scheme of localisation in respect of the projection of the medial geniculate body on the area temporalis has been found by Meyjes (1932) in the rabbit, and more recently Waller (1934), in the rat, has noted a similar type of localisation.

The pulvinar. In a previous series of experiments involving lesions in the parietal lobe of the brain (Clark and Boggon, 1935), no definite cellular degeneration could be detected in the main element of the pulvinar. It was concluded that the main part of the projecting pole of the pulvinar in the monkey has little if any direct connection with the parietal cortex. This conclusion was somewhat unexpected. Poliak (1932), however, was led to doubt a cortical connection of the pulvinar in his Marchi studies. In one of his experiments, in which a lesion destroyed a considerable part of the pulvinar and evidently interrupted the great majority of its dorsal and lateral fibre connections, he records that "from the pulvinar proper, especially from its dorsal portion, only fine, poorly myelinated fibres arise". He further states that "scarcely any of these fine fibres reach the cerebral cortex. However, the cortex might be reached by a few fibres of medium size from the ventral portion of the pulvinar."

In Exps. A and B sharply localised patches of total cell atrophy and gliosis were found in the pulvinar, involving its main element and also the inter-geniculate nucleus. The circumscribed character of this atrophy must be regarded as evidence that the projection of the pulvinar on to the cortex is as precise and localised as it is in the case of other thalamic elements such as the lateral geniculate body and the ventral nucleus. The position of the cortical lesions in these experiments shows that the pulvinar projects (in part at least) on to an area immediately adjacent to and contiguous with the auditory area. The absence of any degeneration in the thalamus following lesions placed more rostrally (Exps. C and E) indicates that the projection area of the pulvinar is probably immediately caudal to the auditory cortex. There is little doubt that this area corresponds to the posterior Sylvian receptive region described by Poliak. This receptive region extends back from the auditory area along the walls and lips of the Sylvian sulcus as far as its caudal extremity. Poliak noted the termination of degenerating fibres here after lesions involving the internal capsule, but was unable to determine their origin. He surmised the probability that they are derived "from the internal geniculate body or from the ventral thalamic region or even from the hypothalamus". The experiments recorded here indicate that they are in reality derived from the pulvinar. Whether the whole of the pulvinar projects on to this posterior Sylvian region can only be decided by making a local excision of this area and studying the

ensuing cellular atrophy in the thalamus. This question is now being undertaken in my laboratory in connection with a more detailed study of the precise projection of the pulvinar. The atrophy in the pulvinar in Exps. A and B involved not only the main element, *pβ*, but also the intergeniculate nucleus, *pd*. In the latter element the area of atrophy was small and localised. In previous experiments (Clark and Boggon, 1935) atrophy in the intergeniculate nucleus of a fairly extensive nature was observed in two cases. It was suggested from the position of the cortical lesion in these cases that the nucleus might project on to Brodmann's areas 18 or 19, or both. The objection to this inference is that Poliak, after extensive lesions in the caudal part of the internal capsule, failed to find any afferent fibres terminating in these areas by the Marchi method. Moreover, Exp. D of the present series, in which a considerable portion of areas 18 and 19 was involved by the lesion, but in which the posterior Sylvian region escaped injury, produced no degeneration at all in the thalamus. Reference to the details of the experiments of Clark and Boggon in which atrophy in the intergeniculate nucleus ensued shows that in both cases the posterior end of Poliak's posterior Sylvian receptive region was also involved, and Exps. A and B of the present series makes it almost certain that this was the source of the atrophy.

The immediate proximity of the pulvinar projection area of the cortex to the auditory area is of some importance to note in connection with degeneration experiments in lower Mammals. Meyjes (1932) includes in his description of the medial geniculate body of the rabbit an element (which he terms the *b*-nucleus) which, as pointed out previously, is probably the homologue of the main element of the pulvinar of the higher Primates (Clark, 1933). He found that secondary degeneration occurred in this element after lesions involving the area temporalis of the cortex (which includes Brodmann's areas 20, 21 and 22). He further notes that it probably "receives fibres mainly or exclusively from the upper corner of the area temporalis". This region of the cortex in the rabbit evidently corresponds to at least a part of the posterior Sylvian receptive region of the monkey's brain.

Nucleus ventralis, pars arcuata. Although this element and the next one to be discussed, the dorso-medial nucleus, are not connected with the temporal lobe, the experiments here recorded have resulted in cell atrophy affecting them of sufficient interest to deserve attention here.

The pars arcuata of the ventral nucleus is a particularly well-defined element in the macaque thalamus, and it is clearly circumscribed except at its dorsal extremity. It has been supposed that it receives predominantly trigeminal fillet fibres and projects on to the face and arm area of the post-central gyrus. However, previous experiments (Clark and Boggon, 1935) suggested that it projects only on to the pre-central gyrus, and this was held to be of some significance in view of the experimental data indicating that this element does not receive any contributions from the fillet system but is rather the terminal station of the thalamic fibres of the superior cerebellar peduncle.

It is interesting to note, therefore, that in Exps. C and E in which the cortical lesion had involved the lower end of the motor cortex (while leaving the post-central cortex intact), sharply localised patches of complete cellular atrophy were found in the ventro-medial extremity of the arcuate nucleus. In these areas the cells have entirely disappeared, and in each case they extend in this position through almost the whole antero-posterior extent of the nucleus. It is to be observed, again, that the circumscribed character of this degeneration suggests that the projection of the arcuate nucleus on to the motor cortex is of a very precise and localised nature.

Nucleus dorso-medialis. In Exp. E the cortical lesion, besides involving the lower end of the motor cortex, extended forwards into the caudo-ventral margin of the granular cortex of the frontal area. It has been previously demonstrated that this granular cortex is the projection area of the dorso-medial nucleus of the thalamus (medial nucleus of human anatomy). This conclusion receives corroboration from the present experiment in which a sharply localised patch of complete cellular atrophy was found in the ventro-medial part of the dorso-medial nucleus. The circumscribed character of this degeneration suggests that different sections of the nucleus project on to corresponding different sections of the area frontalis in quite a precise and localised manner.

The thalamic connections of the cortical areas of the temporal lobe

In these experiments evidence of ascending thalamic connections with the temporal lobe has only been found for cortical area 22. Exps. C and E indicate that approximately the rostral 2 cm. of this area do not receive fibres from the thalamus. Exp. D leads to the same conclusion regarding the posterior third of the exposed portion of the area. The auditory and pulvinar projection fibres probably project almost entirely on to that part of area 22 which is submerged in the lower wall of the middle and caudal parts of the Sylvian fissure. This is in accord with the evidence adduced by Poliak from his Marchi studies.

The posterior end of area 21 was involved by a cortical lesion in Exp. D and produced no cellular atrophy in the thalamus. Area 20 was not injured in any of the experiments. Poliak's observations make it practically certain that neither of these cortical areas receives thalamic fibres in any part of its extent. This may also be inferred to be the case by a process of exclusion. In this laboratory almost every thalamic element which has cortical connections has now been related to one or other of the cortical areas, and there is practically nothing in the thalamus left over which might comprise a possible source of fibres to areas 21 and 20.

It is rather remarkable that a very great proportion of the cortex of the temporal lobe apparently receives no fibres from the thalamus. In this respect the temporal lobe stands in strong contrast with the other lobes of the cerebral hemisphere. According to Poliak's description, a small part of the exposed cortex of the parietal lobe is similarly unconnected with the thalamus, and

his experiments indicate that the same applies to the peristriate areas of the occipital lobe. In the frontal lobe, the motor, pre-motor and frontal areas all receive contributions from thalamic nuclei.

SUMMARY

A study has been made of secondary cell degeneration in the thalamus following local cortical ablations in the temporal lobe in the macaque monkey. The auditory projection area is limited to the middle third of the superior temporal gyrus. The pulvinar projects, in part at least, on to the posterior Sylvian receptive region of the cortex. Lesions involving the pre-central convolution and the area frontalis granularis lead to localised cell atrophy and gliosis in the arcuate nucleus and the dorso-medial nucleus respectively.

The cost of the animals used in this research was met by a grant from the Royal Society, which I wish gratefully to acknowledge. I am also indebted to my laboratory technician, Mr E. Thompson, for the skill he has shown in the preparation of large numbers of serial sections, and to Mr Chesterman, of this department, for the care he has taken in the microphotography.

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EXPLANATION OF PLATES I AND II

Plate I

Fig. 1. Vertical section through the lesion in Exp. B. *Syl.* Sylvian sulcus. *Par.* Parallel sulcus. The figure 21 refers to Brodmann's cortical area. ×8.

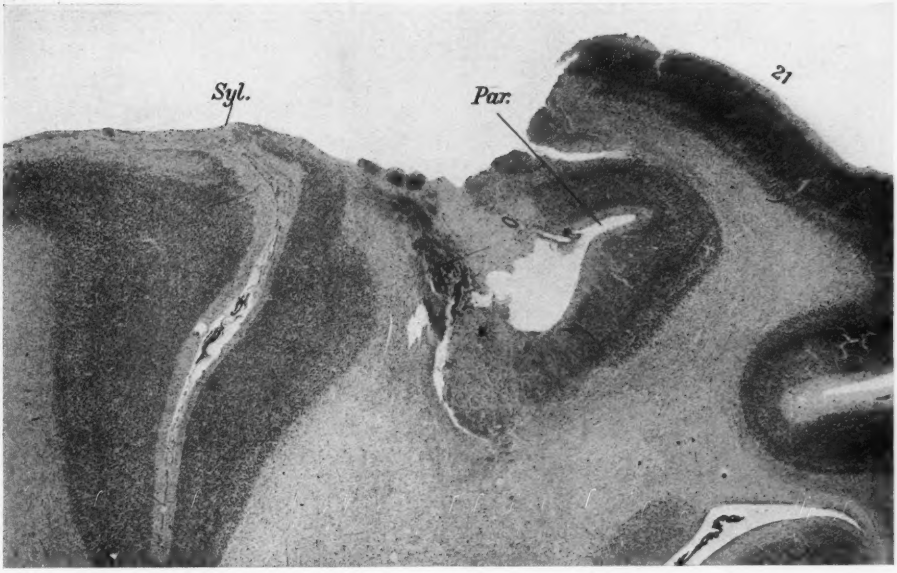
Fig. 2. A. Transverse section through a normal medial geniculate body in *Macaca*. ×18. B. Transverse section through the medial geniculate body in Exp. B showing the area of cell degeneration following a lesion in the middle of the superior temporal gyrus. ×18. *Pd.* Nucleus intergeniculatus. *Pg.* Nucleus parageniculatus.

Plate II

Fig. 1. Transverse section through the pulvinar in Exp. A, showing the extent of the cell degeneration (cf. text-fig. 2 C). ×22.

Fig. 2. Transverse section through the middle of the thalamus in Exp. C, showing the sharply circumscribed area of cell atrophy and gliosis in the medio-ventral part of the arcuate nucleus (cf. text-fig. 6). ×22. *Cm.* Centre median nucleus. *Va.* Nucleus ventralis, pars arcuata. *Ve.* Nucleus ventralis, pars externa.

Fig. 3. Transverse section through the middle of the thalamus in Exp. E, showing the areas of cell atrophy in the arcuate and dorso-medial nuclei (cf. text-fig. 9). ×17. *Cm.* Centre median nucleus. *Dm.* Dorso-medial nucleus. *Va.* Nucleus ventralis, pars arcuata.



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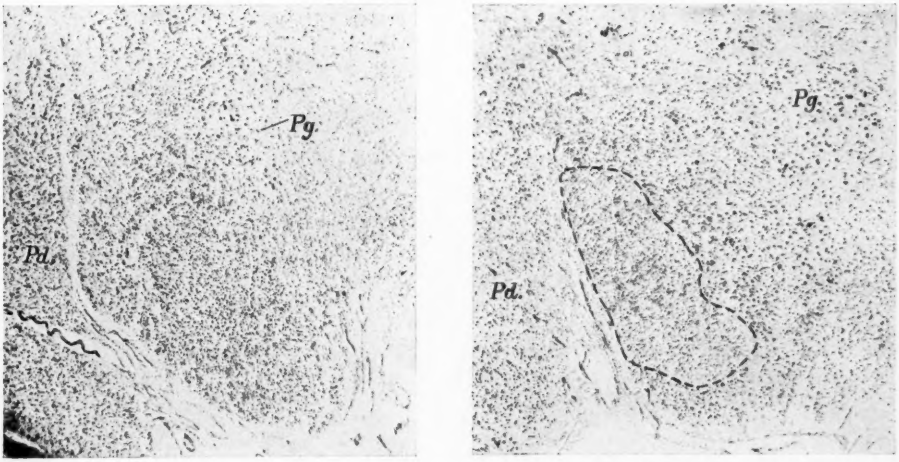

A Fig. 2. B

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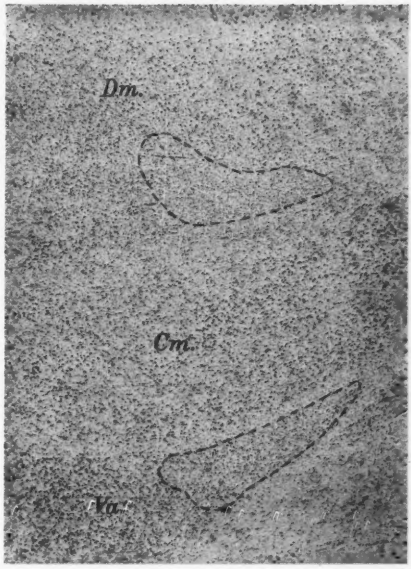
Fig. 1.



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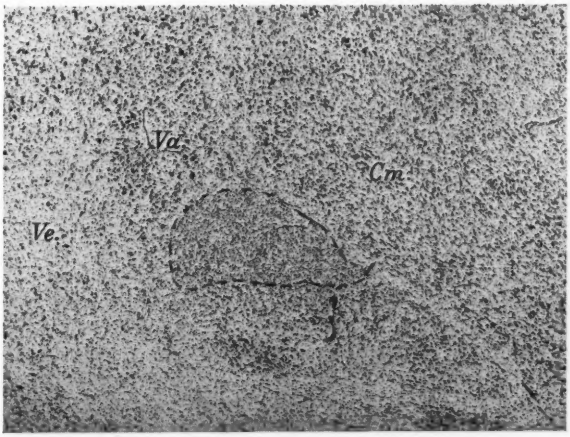

Fig. 2.

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A PRELIMINARY NOTE ON THE PRESENCE OF DOUBLE CONES AND OIL DROPLETS IN THE RETINA OF MARSUPIALS

By KEVIN O'DAY

Melbourne

Remarkably little work has been published on the retina of Marsupials and Monotremes since Hoffman (1) in 1876 reported the presence of oil droplets in the cones of *Macropus giganteus* and *Wallabia bennetti*, and Marcus Gunn (2) in 1884 described similar structures in the cones of *Ornithorhynchus*. There is a rich field for investigation in the visual organs of these lower members of the mammalian family, and a preliminary investigation of the eyes of *Dasyurus viverrinus* (Shaw) immediately revealed features of interest.

Dasyurus viverrinus (8) is a small carnivorous Marsupial to be found in an area extending from Southern Queensland to South Australia. Its habits are mainly nocturnal, although it will venture out for food in the daytime.

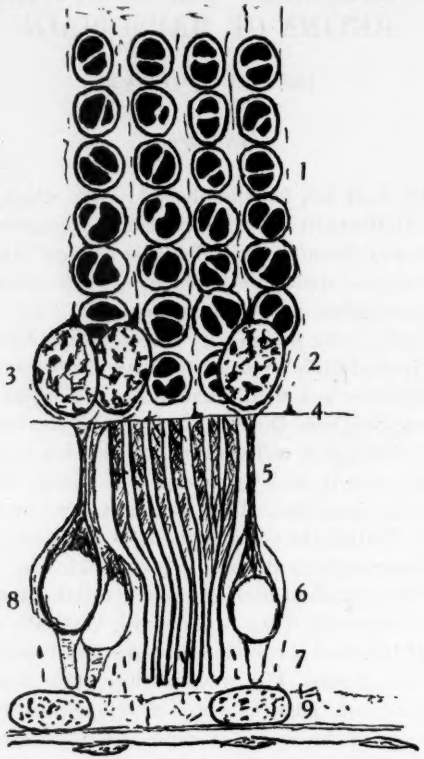
The eyes are prominent, and the visual axes widely divergent. The dark brown iris is heavily pigmented. In a well-lighted room the vertically oval pupil is moderately dilated, the vertical diameter being very little greater than the horizontal. Under such conditions, it is quite an easy matter to examine the fundus of the living animal with an electric ophthalmoscope. In brilliant sunshine, the pupil contracts down to a narrow, vertically oval slit.

Examination of the fundus reveals a well-marked tapetum confined to the upper half of the eye-ground. The greyish white optic disc is situated in the upper temporal quadrant. There is a well-developed central retinal vascular system. There is no ophthalmoscopic evidence of a fovea, although the arrangement of the vessels suggests the demarcation of a central strip. The general picture of the fundus is not unlike that of the Virginian opossum (*Didelphis*) as figured by Lindsay Johnson (5).

Two methods of fixation were employed for the investigation. One animal was injected with Kolmer's fluid, and the eyes, after being embedded in celloidin, sectioned and stained with haematoxylin and eosin, Mallory's triple connective-tissue stain (3, 4), and with Heidenhain's iron haematoxylin. An eye from another animal was fixed in osmic acid and sectioned in paraffin. Of the stains employed, that of Mallory, as pointed out by Walls and Mayou, is of great value for the investigation of the structure of the outer members of the rods and cones.

The outer nuclear layer of the retina conforms to the mammalian type.

In the central area it is eight to ten nuclei thick. The chromatin in the majority is arranged in two, and occasionally three, densely staining, bean-shaped masses (Plate I, fig. 2). Arranged at intervals along the external limiting membrane are larger nuclei in which the chromatin is scattered throughout the nucleus in small masses connected by a fine network. Many of these larger



Text-fig. 1. Semi-diagrammatic reconstruction of the outer layers of the retina of *Dasyurus viverrinus*. 1, rod nucleus; 2, cone nucleus; 3, double cone nuclei; 4, external limiting membrane; 5, cone myoid; 6, cone ellipsoid with oil-droplet and mantle; 7, cone outer segment; 8, double cone myoids, ellipsoids, oil-droplets and outer segments; 9, retinal pigment epithelium.

nuclei are paired, there being no obvious difference in the size or shape of the members of a pair (Plate I, figs. 1 and 2).

With Mallory's stain, the myoid of the outer member of the cone stains a lighter purple than the ellipsoid, and, in the specimen examined, is long and attenuated (Plate I, fig. 1 and text-fig. 1). The bulbous ellipsoid contains a globular mass which does not stain. A thin mantle, staining a delicate blue, can be made out surrounding the ellipsoid (Plate I, fig. 1 and text-fig. 1).

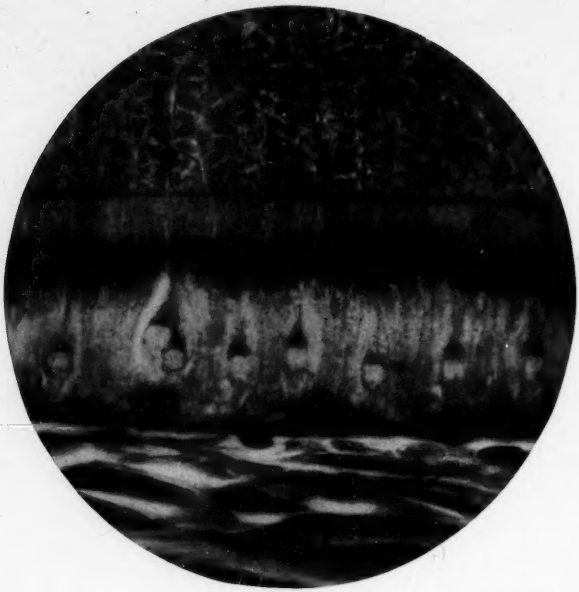


Fig. 1.

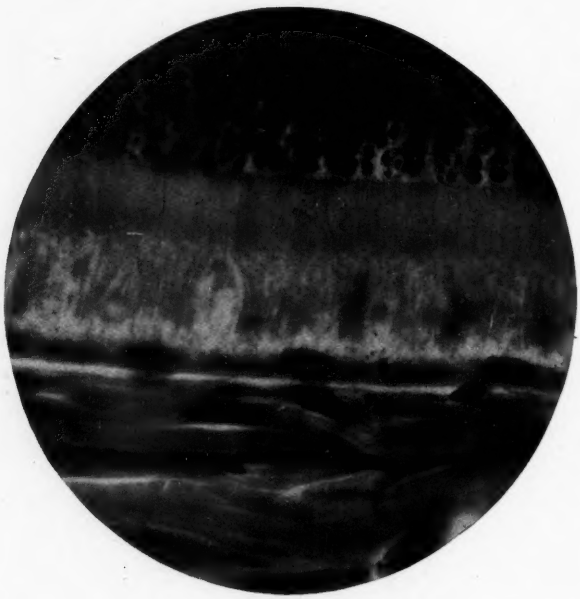


Fig. 2.

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From the distal extremity of the latter projects a short, conical structure staining a slightly deeper blue than the mantle, and presenting a marked contrast with the distal extremities of the rods, which stain a bright orange (Plate I, fig. 1 and text-fig. 1).

Many of the cones are arranged in pairs. Both members are of equal size, each containing the same globular, non-staining mass in the ellipsoid. It is difficult to differentiate the two individual myoids as they run proximally from the double ellipsoids to the double nuclei seated on the external limiting membrane (Plate I, fig. 1).

As the globular mass stains a dense black in the osmic acid preparations, it is reasonable to assume that it is an oil globule.

This preliminary investigation confirms the work of Hoffman with regard to the presence of oil droplets in the cones of Marsupials. It demonstrates also the presence of double cones of the same size and shape in the retina of *Dasyurus viverrinus*. There is no previous record of their presence in Mammals (3, 6, 7). They are present in the retina of all other Vertebrates.

Finally, it is of interest to note that an examination of the retina of *Pseudochirus laniginosus* (the ring-tailed opossum), undertaken at the same time, confirms the above observation by revealing both double cones and oil droplets.

The work is being undertaken in the Anatomy Department of the University of Melbourne, at the suggestion of Prof. Wood Jones. I wish to thank him for the facilities he has so generously placed at my disposal, Sir James Barrett for his kindly interest and many valuable suggestions, Dr Edward Ford for his help in searching the literature, and Mr H. Marriott for his skill in preparing the sections and photographs.

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EXPLANATION OF PLATE I

Fig. 1. Retina of *Dasyurus viverrinus* showing nuclei and outer members of rods and cones. Mallory's triple connective-tissue stain.

Fig. 2. Retina of *Dasyurus viverrinus* showing the difference in structure between the nuclei of the rods and cones. Heidenhain's iron-haematoxylin.

THE INTRANEURAL PLEXUS AND ITS SIGNIFICANCE

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THE arrangement of the fasciculi within a nerve trunk is a subject of both theoretical and practical importance, yet the present state of knowledge in this connection, as evidenced by references to it in the consideration of nerve injuries and their treatment, appears to be very incomplete. This is so in spite of the fact that within the last twenty years a considerable literature dealing with the problem has accumulated. The work which has previously been carried out will first be reviewed briefly and in this manner the current views on intraneural topography will be outlined.

As was quite natural it was at first thought that nerve trunks were composed of numbers of parallel fasciculi without any intercommunicating bundles of nerve fibres passing between these. It was believed that any small branch arising at the peripheral end of a large nerve trunk could—without any division of nerve fibres—be stripped away from the trunk until its origin from a single or several spinal nerve roots had been attained. Indeed it would appear that this method of stripping up peripheral branches was made use of in the original determination of the segmental supply of muscles. This opinion was strengthened by the work of Stoffel (20, 21). He speaks of the "cable structure of nerves", and definitely states that the fasciculi in the proximal portion of a nerve trunk are the upward continuations of its peripheral branches. After previous examination in a corpse it was therefore possible to recognise high up in a nerve trunk at operation the fasciculi which corresponded to a particular peripheral branch, and on this view was based Stoffel's operation of motor neurectomy for spastic paralysis and also certain nerve-grafting operations for the residual palsies in cases of anterior poliomyelitis. That the former of these operations is now widely performed is due not to the correctness of Stoffel's anatomical opinions but to the fact that as it is now performed it consists in the division of the actual branches of a nerve trunk to certain muscles. No attempt is made to attack the fasciculi above the true origin of the branch concerned. Further reference will be made to this point at a later stage. Marie and his co-workers (13) made observations which were thought to support the idea of a simple intraneural topography. By the electrical stimulation of nerves exposed in the course of some surgical operation it was shown that different muscles could be caused to contract by stimulating different points on the nerve. This had its importance in showing that at a

particular level the motor fibres supplying a certain muscle are, to some extent at least, associated with one another. It gives no information, however, as to the position occupied by these fibres in their further course through the length of the nerve to the periphery, and therefore, in spite of statements to the contrary, does not necessarily support Stoffel's view as to the cable structure of nerves. Putti and others also believed in the maintenance of a simple fascicular pattern.

At the beginning of the Great War, therefore, the general belief was in favour of a very simple intraneural topography. Early in the century, however, Bardeen had demonstrated the anastomoses of fasciculi occurring in nerve trunks, and with the stimulus of large numbers of nerve injuries resulting from war wounds other workers soon confirmed his results and the earlier views could no longer be readily accepted. Heinemann (9) demonstrated anastomosing and branching fasciculi in the median, ulnar and radial nerves. Langley and Hashimoto (12) demonstrated the complex intraneural plexus which exists in the sciatic nerve. They remarked upon the fact that the plexus appeared to be more complex immediately proximal, and less complex, immediately distal to the points of the origins of the main branches from the nerve. Dustin (6) demonstrated, by means of serial sections, the frequent changes in fascicular topography which occur from the proximal to the distal end of a nerve trunk. For this work he used the large nerve trunks of the upper limb. McKinley (14), working on the sciatic nerve, produced further evidence of the complexity of intraneural topography. His methods included dissection, and partial section of nerves in living animals with later examination of the trunks to discover the distribution of the areas of degenerated nervous tissue. He also made use of electrical stimulation of the divided sciatic nerve. His results here differ from those of Marie described above in that he finds that following the separation of the distal cut end into a number of groups of nerve fibres stimulation of one of these groups gives rise to contraction of all the muscles supplied by the nerve trunk, i.e. according to this result the motor fibres to a particular muscle are not grouped together in the nerve but are widely separated from one another by other fibres.

No satisfactory explanation of the significance of the intraneural plexus has so far been forthcoming. As will be seen later this plexus formation occurs in unisegmental nerves. It therefore cannot be purely a matter of segmental rearrangement of fibres. Langley and Hashimoto alone have attempted to explain the presence of the plexus. They suggest that just as the lumbo-sacral plexus collects together fibres from various spinal segments to form nerves for the supply of large areas, so perhaps the intraneural plexus continues this grouping within these nerve trunks in order that each of their branches may contain fibres from the requisite segmental sources. For the reason mentioned above it is considered that this is not a sufficient explanation. The work carried out for the present investigation will now be described.

MATERIAL AND METHODS

Several methods have been used in the investigation of this problem. Included among these are the methods of dissection, examination of serial sections of nerve trunks, tracing of areas of degeneration through nerves after partial section in living animals, and also electrical stimulation of localised areas on nerve trunks in Man and animals. In so far as the present investigation is concerned the method of dissection was employed because it was thought desirable to establish how far intraneural plexus formation was a property of all the peripheral nerves of the body. It was hoped that such an examination—as complete as possible—might enable one to understand the factors which give rise to these plexuses. Hitherto the investigations have been confined to the large nerve trunks of the limbs, and this may account for the obscurity in which the significance of this plexus formation is shrouded. Some workers have investigated the nerves of the upper limb, but many others have concentrated upon the sciatic nerve. From its very size it is difficult to examine this satisfactorily, and when it is remembered that the sciatic nerve corresponds to the median and ulnar and part of the radial nerve in the upper limb it will be realised that a search here for the explanation of intraneural plexus formation is likely to prove fruitless. In carrying out the investigation here to be described it was hoped that by the examination of certain smaller and simpler nerves more useful information might be obtained. This work will now be described.

Before proceeding to a consideration of the method in more detail it may be well to enumerate the different nerves examined, since the ultimate conclusions depend upon the correlation of the results of dissection with the type of nerve examined:

(1) Non-segmental unifunctional nerve—optic nerve.
(2) Uni-segmental plurifunctional nerve—an intercostal nerve.
(3) Non-segmental plurifunctional nerve—the trigeminal nerve.
(4) Non-segmental motor nerve—facial nerve.
(5) The smaller plurisegmental nerves:
    (*a*) Sensory: posterior cutaneous nerve of thigh.
    (*b*) Motor:
        (i) Long thoracic nerve.
        (ii) Branches of median nerve to flexors of wrist and fingers.

(6) Brief examination of some of the larger plurisegmental nerves was made (e.g. median and ulnar nerves in upper arm). This revealed a plexus so complex as to appear meaningless, so that the remainder of the work was concentrated on simpler nerves.

As will be described later a certain number of ordinary dissections were made to obtain first-hand information on the macroscopic anatomy of particular nerves—especially where some differences of opinion existed as to what constituted the most accurate anatomical description of the nerve concerned.

The larger part of the work however consisted in the examination of nerves which had been excised entire following their ordinary dissection. These were immersed in various solutions in order to simplify subsequent unravelling. Of these solutions various strengths of nitric acid were employed, but in the majority of cases a 33 per cent. solution of spirit was used. Following a week or more of such treatment the nerve was pinned out on a sheet of cork—usually under water—and with a dissecting microscope giving a magnification slightly greater than ten times, the fibrous sheath was dissected off and an attempt made to define and separate the nerve bundles. Mounted Hagedorn needles were largely used in this procedure. With one exception—to be noted later—it was in this way found possible to carry the dissection to the approximately same stage in each case—a most important point if the results are to be comparable. Working at this magnification it was possible to be certain that in separating nerve bundles and removing intervening fibrous tissue no important connecting fasciculi were inadvertently removed—that is, where a plexus was present it was seen and not destroyed.

Photographs of the completed dissections were made and considerably enlarged. In a few cases, where for some reason photography failed to demonstrate the essential features of the specimen, drawings were made use of instead.

THE DISSECTIONS

(1) *The optic nerve*

Brouwer (25) and others have carried out experiments on the localisation of the retina within the optic system, and as a result of this work it has become clear that this functional localisation is a most exact one. One would therefore expect that the nerve would be composed of easily separable parallel fasciculi.

Difficulty was encountered in making this dissection, for it was found that the optic nerve in both foetus and adult dissecting-room subject was usually poorly preserved. This condition became more marked as the chiasma was approached. In spite of this it was found that the main fascicular groups could be separated with comparative ease, and their parallel arrangement was obvious (fig. 1). It will be noticed, however, that the dissection could not be carried to as complete a stage as in other nerves. It will thus be seen that the simple (single) function of the optic nerve is associated with a simple parallel arrangement of its fasciculi. The latter arrangement is also what would be expected from the results of experiments on retina localisation within the nerve and from the clinical results of pressure upon it.

(2) *An intercostal nerve*

While the intercostal nerves contain fibres subserving motor and the various so-called common sensations (deep and superficial), yet since they are unisegmental it might be expected that the intraneural pattern would be simpler than that which has been described for the large plurisegmental nerve

trunks of the limbs. Any plexus formation noted in them obviously cannot be concerned with the segmental apportionment of fibres and must therefore be due to some other cause.

(*a*) *Gross anatomy of the intercostal nerves.*

Six of the thoracic segmental nerves were dissected from their spinal origins to their terminal distribution. It was found that each existed as a single trunk from the point at which the posterior primary division arose to

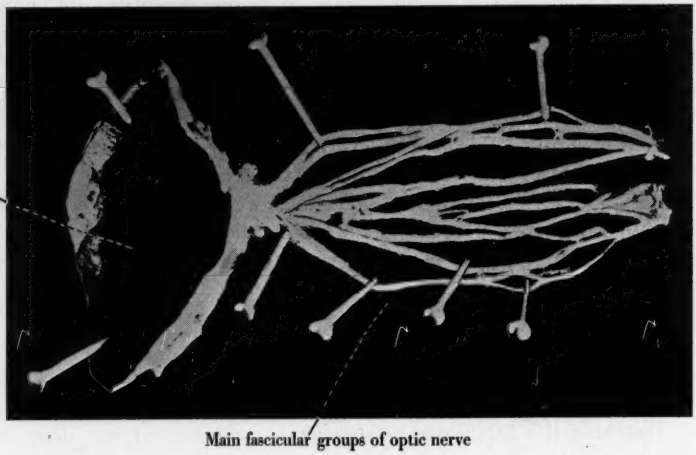

Fig. 1. Photograph of dissection demonstrating the arrangement of the larger fascicular groups in the optic nerve.

the region of the posterior axillary line. As this trunk was traced from its origin distally small muscular branches were seen to arise from it, and at the posterior axillary line arose the large lateral cutaneous branch. Beyond this the intercostal nerve existed as a single trunk and continued its course to the periphery where it terminated as an anterior cutaneous nerve (fig. 2). This description is in accord with that to be found in standard anatomical works, and that put forward more recently by Siddiqui and Mullick (17). However, Davies, Gladstone and Stibbe (4) state that at any point anterior to the angle of a rib the intercostal nerve (so-called) is really three separate nerves—these being the main intercostal nerve, the lateral cutaneous branch and the collateral intercostal nerve, which may rejoin the main nerve or be distributed as an accessory anterior cutaneous nerve. After the intraneural topography of the intercostal nerve has been considered, mention of this matter will be made again, for it is believed that the two accounts are only superficially different from one another.

(*b*) ***The intraneural topography of intercostal nerves.***

The various portions of the nerve will be considered separately.

(i) ***The anterior and posterior nerve roots.*** Each nerve was excised entire together with the segment of the spinal cord, to which it was attached. It was found that the anterior and posterior nerve roots could be separated easily

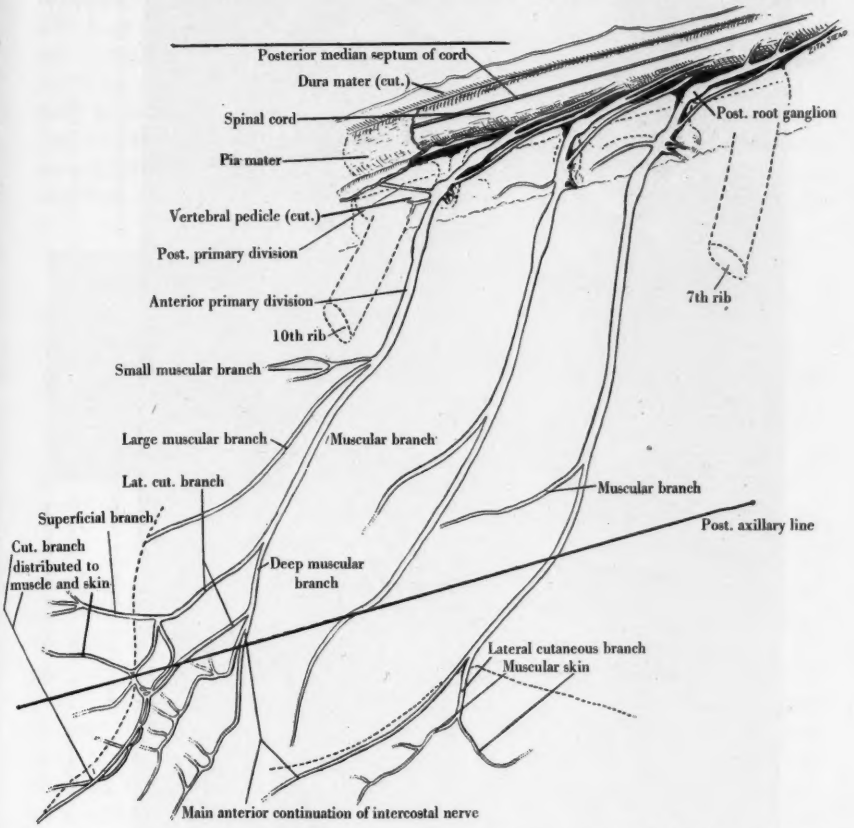

Fig. 2. Diagrammatic drawing of three intercostal nerves demonstrating site of origin of lateral cutaneous branch of each.

into numbers of fine parallel fasciculi throughout their length, there being no suggestion of any plexus formation (fig. 3). The relatively fine connective tissue which surrounds and pervades these roots in their intrathecal course renders the dissection a simple one. It will be noted that this process of separation of the fibre groups in the posterior root was interrupted at the ganglion, and it was not found possible to separate this into groups of cells corresponding to the groups of fibres. Similarly, as one approached the spinal

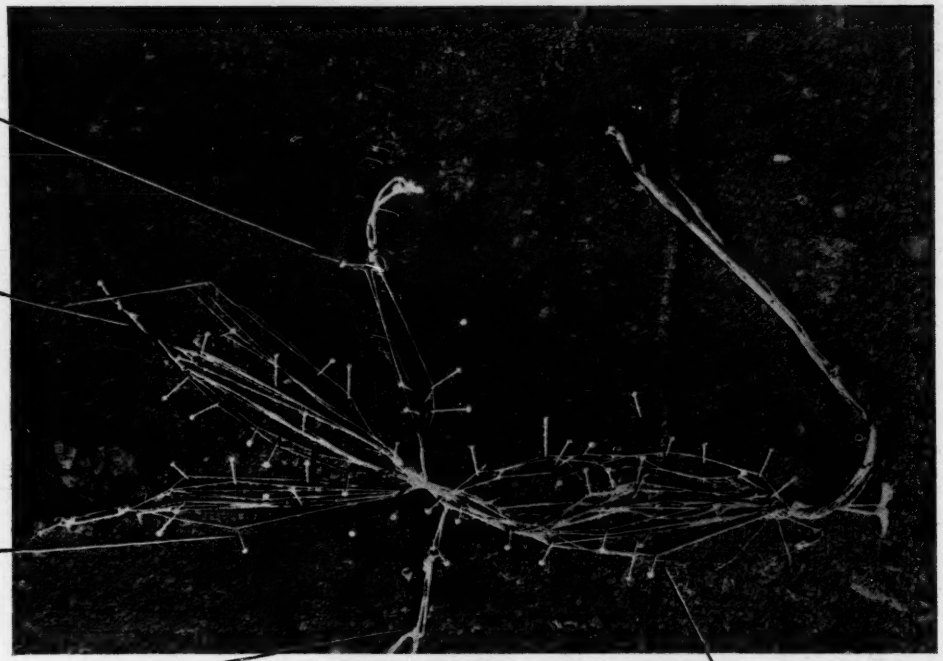
Posterior primary div.
Anterior nerve root (showing parallel fasciculi)
Posterior nerve root (showing parallel fasciculi)
Communicating branches to sympathetic
Intercostal nerve (showing plexus)

Fig. 3. Photograph of dissection of anterior and posterior nerve roots and posterior portion of the thoracic segmental nerve to which they give rise.

cord it was not found possible to trace the fine parallel fasciculi into any special arrangement as they entered its white matter.

(ii) *Intercostal nerve itself.* Following the union of anterior and posterior nerve roots there exists a coarse plexus of intercommunicating nerve bundles within the nerve trunk which they form. This extends distally for a distance of approximately 5 cm. (fig. 3). Beyond this plexus the trunk can be separated into a number of relatively large and parallel fascicular groups—that is to say, there is no gross intraneural plexus (fig. 4). If now each of these larger nerve bundles be examined there will be found at various points in each a finer type of intraneural plexus which involves a regrouping of the smaller fasciculi of which the main bundle is composed. It may indeed even be concerned with the regrouping of nerve fibres themselves. In an intercostal nerve therefore there exists firstly a coarse intraneural plexus concerned in the

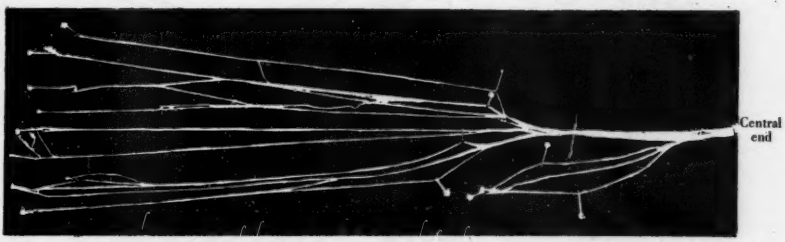

Fig. 4. Photograph of a dissection of an intercostal nerve trunk to demonstrate the parallel arrangement of the main fascicular groups within it.

formation of the larger bundles of fibres and within these bundles finer intercommunications occur, and are probably concerned with the production of the final arrangement of fibres.

(iii) *The sympathetic fibres in the intercostal nerve.* It was found that in every case in the nerves examined the post-ganglionic sympathetic fibres became distributed throughout the intercostal nerve immediately after the grey ramus had united with the main trunk. This is clearly shown in the drawing (fig. 5), and it is obvious that the occurrence is one factor in the production of the intraneural plexus in the proximal part of the intercostal nerve trunk. This finding has its interest in connection with the explanation of the vascular symptoms in cases of cervical rib. Telford and Stopford (22) state that sometimes the post-ganglionic fibres entering the first dorsal nerve may remain grouped together and run towards the periphery for some distance as a separate fasciculus within the sheath of the main nerve trunk. It was thought that the vascular symptoms in cases of cervical rib might be due to direct stimulation of this fasciculus, which was described as being situated in the most inferior portion of the nerve. Later Blair, Davies and McKissock (1) examined the brachial plexus from a case in which the vascular symptoms

had been very marked. They agree with Stopford and Telford's suggestion that the symptoms are due to sympathetic irritation, but believe that this is brought about by fibroses in the trunk at the point of friction and not to an asymmetrical arrangement of sympathetic fibres. Although the first dorsal nerve was not examined here, in the case of the lower nerves an early distribution of sympathetic fibres throughout the main trunk has been shown.

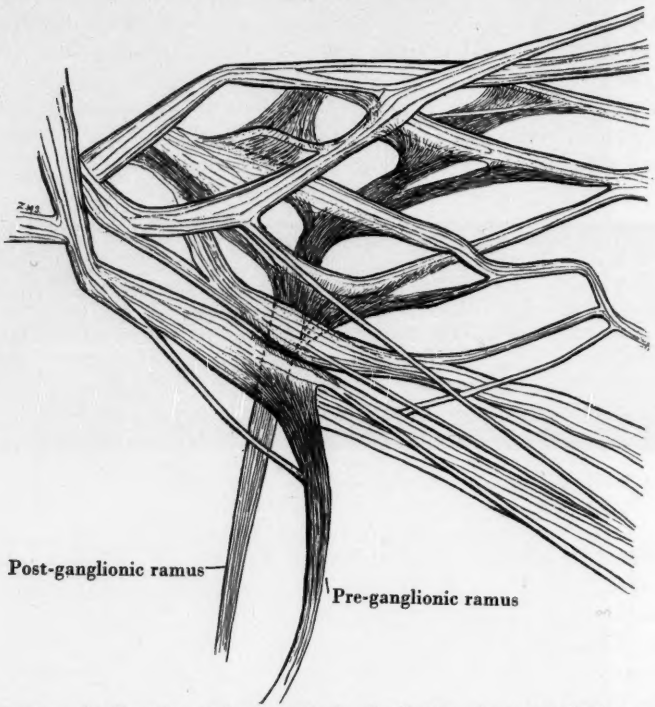

Fig. 5. Drawing of a dissection to demonstrate the distribution of post-ganglionic sympathetic fibres within the proximal portion of an intercostal nerve trunk.

(*c*) *The relationship of their intraneural topography to the gross anatomy of the intercostal nerves.*

On examination of the pinned-out intercostal nerve (fig. 6) it will be noticed that following the union of anterior and posterior nerve roots and for the distance of approximately 3 cm. distal to this the nerve trunk is somewhat constricted, while beyond this region it is considerably broader. There is also a difference in consistency to be noted, the narrow part being firm to touch, while the wider portion is soft and of looser texture. As the anterior and posterior roots were being excised from within the dural theca it was necessary to remove a portion of the dura surrounding these roots as they approached

and fused with one another. This dural sheath was later dissected off and was found to extend forwards as a fibrous membrane to the anterior extremity of the narrow portion of the nerve trunk. Anterior to this region the nerve sheath was looser and infiltrated with a quantity of fat. The constricted portion of the nerve approximately corresponds to that which contains the coarse intraneural plexus, while the wider portion has been found to consist of large parallel fasciculi. It may therefore be said that the true origin of the lateral cutaneous and collateral intercostal nerves is in the proximal part of the intercostal nerve trunk. The point of superficial origin of the branches is variable, however, and indeed the fasciculi of the so-called collateral intercostal nerve may never separate from those of the main nerve, or, if they do separate from them, union of the two groups may occur again at a later stage in their course. The factors determining the superficial origins of these branches are not understood, but it is suggested that the quantity of fat in the sheath may be one.

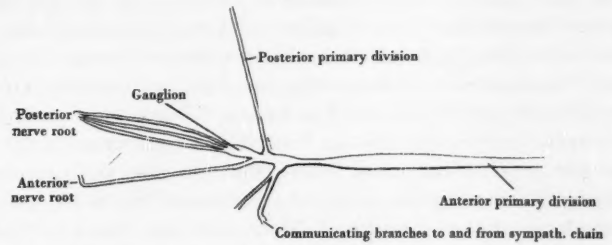

Fig. 6. Diagrammatic drawing of posterior portion of a spinal (intercostal) nerve with its roots of origin.

(3) *The trigeminal nerve*

In such an investigation as this the intraneural topography of the fifth cranial nerve is of considerable importance. From the point of view of comparative anatomy which reveals the trigeminal as one of the oldest and most primitive of the sensory nerves it might be expected that its intraneural pattern would enable one to understand that of other peripheral sensory nerves. Moreover, the results of surgical interference with the peripheral branches, ganglion and central root have concentrated attention on the importance of the intraneural arrangement of fibres.

(*a*) *The sensory root.*

As a result of many investigations it is now known that the ophthalmic, maxillary and mandibular divisions of the trigeminal nerve are separately represented within the nucleus of the tractus spinalis—the third division terminating in its proximal portion, the ophthalmic in its caudal portion, and the maxillary division intermediately. Spiller (18) discusses the experimental work on the subject which had at that time been performed by others and

himself. Eisenholer and Wallenberg produce clinico-pathological evidence on the same matter. Further, in the nucleus of the tractus spinalis pain and temperature sensations are preponderantly represented. At some point between the ganglion and their final destination a rearrangement of fibres must therefore occur. Surgical experience has given rise to two views as to the site of this rearrangement. On the one hand it is suggested that the rearrangement occurs within the brain stem or spinal cord, on the other (Dandy) it is believed to occur in the sensory root of the trigeminal nerve itself.

The fact that the peripheral branches of the fifth nerve are separately represented (as regards some of their functions at any rate) within the nucleus of the tractus spinalis would lead one to expect that the fibres from each of these branches would remain grouped together within the sensory root. The correspondence of the results of division of certain of the peripheral branches of the nerve with those of partial excision of the Gasserian ganglion and various partial sections of the sensory root must lend considerable support to this view. But there is still further evidence on this subject. Morphologically it has long been known that the ophthalmic division of the nerve and its associated portion of the ganglion is, in early developmental stages, widely separated from the remainder of nerve and ganglion, and in some animals the separation persists into adult life. Frazier and Whitehead (8) confirmed these observations and pointed out that the fasciculi in the sensory root were parallel to one another in their backward course from the ganglion to the pons—no intermingling of fibre bundles occurred. Davis and Haven (5) described the surgical anatomy of the sensory root. They point out that a rotation occurs in the root as it passes centrally, so that fibres which at the ganglion are situated laterally and inferiorly are placed laterally and superiorly at the pons, while the superior and medial fibres at the ganglion are displaced to an inferior and medial position at the pons. These workers remark upon the anastomoses of fasciculi near the hilus of the ganglion, and on the whole are of opinion that the fibre bundles of the sensory root do not run a parallel course.

The results of operative experience in dealing with large numbers of partial sections of the sensory root have strengthened the opinion that the three peripheral branches of the fifth nerve are separately represented in the central root. Frazier (7) and Jefferson (10) are of this opinion, although the latter believes that some interweaving of fibres does occur. It is suggested that the ophthalmic division is represented in the superior and medial portion of the root, the mandibular division in its inferior and lateral portion, while the maxillary division occupies an intermediate position. Dandy (3), however, from a consideration of the results of partial section of the sensory root close to the pons (following the subcerebellar approach) is of opinion that there is no accurate subdivision of the root into three anatomical divisions corresponding to the peripheral branches. From the fact that in these cases the pain of trigeminal neuralgia has been cured without the production of any demonstrable anaesthesia by the section of a selected portion of the root, he

suggests that there must be a rearrangement according to function of fibres in the sensory root. Wilkins and Sachs (24), from the areas of anaesthesia produced by partial sections, believe that an interweaving of fasciculi must occur in the central root. Van Nonhuys (23), from anatomical studies, is of opinion that the sensory root is not composed of three parts corresponding to the peripheral divisions of the nerve. For this reason he states the operation of partial section of the sensory root is not based on anatomical facts and is therefore an unreliable procedure.

Several dissections were made to display the semilunar ganglion with its central root and peripheral branches, and photographs and a drawing of three of these specimens are seen in figs. 7, 8 and 9. The rotation of the fibres of the central root as it approaches the pons was clearly made out and is seen in fig. 9. It will be seen that the most inferior fibres in the sensory root at the level of the ganglion incline upwards as the pons is approached, so that at the point of entrance of these inferior fibres they have become displaced laterally in relation to the other fibre groups. In the same way the most superior fibres incline downwards so that at the pons they are medial and superior to the other fibres. The intermediate fibres in the anterior portion of the sensory root maintain this intermediate position at the pons. (This is an arrangement similar to that described by Davis and Haven, but the rotation observed by me was less than theirs.) A superficial examination of these dissections reveals the fact that the central root contains numbers of parallel fasciculi (figs. 7 and 8). These photographs were taken after the region had been exposed and before any attempt had been made to separate the fascicular groups from one another.

Following excision of the nerve and its treatment in the usual manner the motor root was found to consist of easily separable parallel bundles. The photograph in fig. 10 shows a dissection of the sensory root. It consists of a series of parallel bundles of nerve fibres, and any apparent union of fasciculi could be shown to be artificial in that the bundles were easily separable from one another, and simply contained within a single fibrous sheath. Close up to the ganglion and especially in connection with the fibres in the central portion of the root the arrangement was more complex, several well-marked intercommunications of the type referred to above being present. It should be pointed out, in connection with the intercommunications occurring here and in the remainder of the root, that these are such as to involve the grouping together of neighbouring fasciculi only, e.g. no case of fasciculi passing from the upper portion of the root to unite with fasciculi in its lower portion, or *vice versa*, was noted. A few such local and apparent communications (which have been purposely left unseparated) are to be seen in fig. 10. The local character greatly diminishes their importance, as it implies that the upper, middle and lower portions of the sensory root can be considered to be isolated bundles of nerve fibres. From an examination of the specimens it would appear justifiable to state that the superior portion of the sensory root is concerned

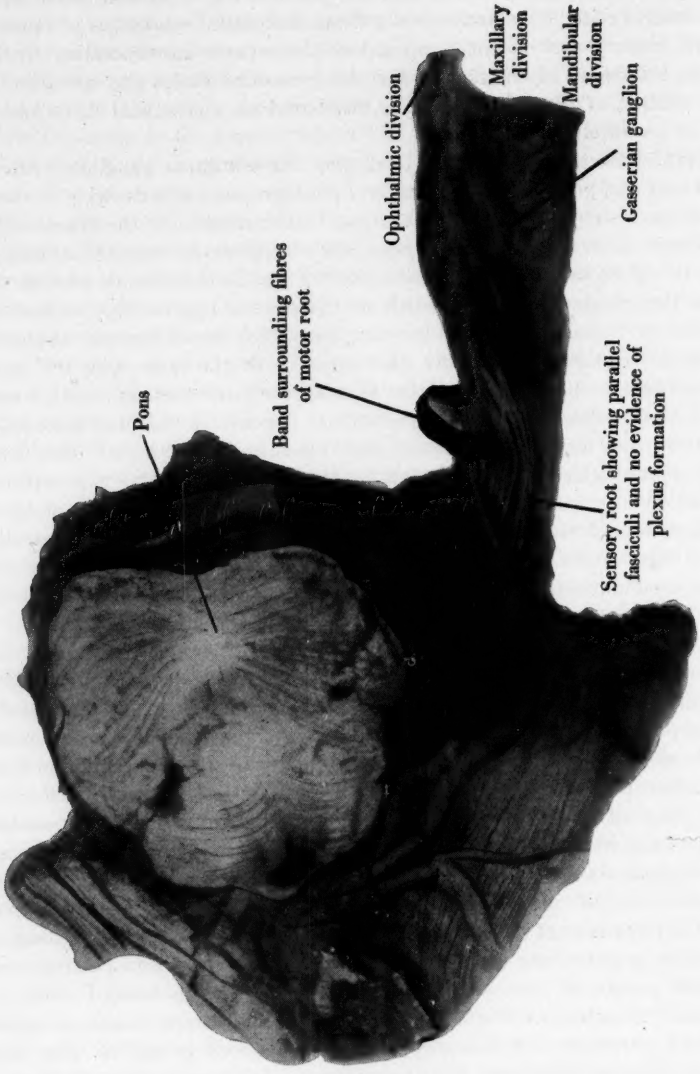

Fig. 7. Photograph of a dissection demonstrating semilunar ganglion, and central roots of trigeminal nerve (superior aspect).

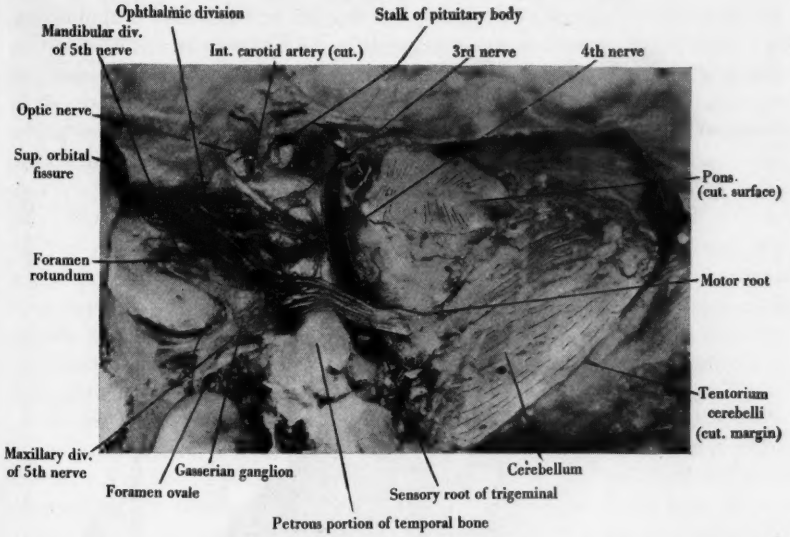

Fig. 8. Photograph of a dissection demonstrating peripheral branches of trigeminal nerve, its ganglion and central roots (lateral aspect).

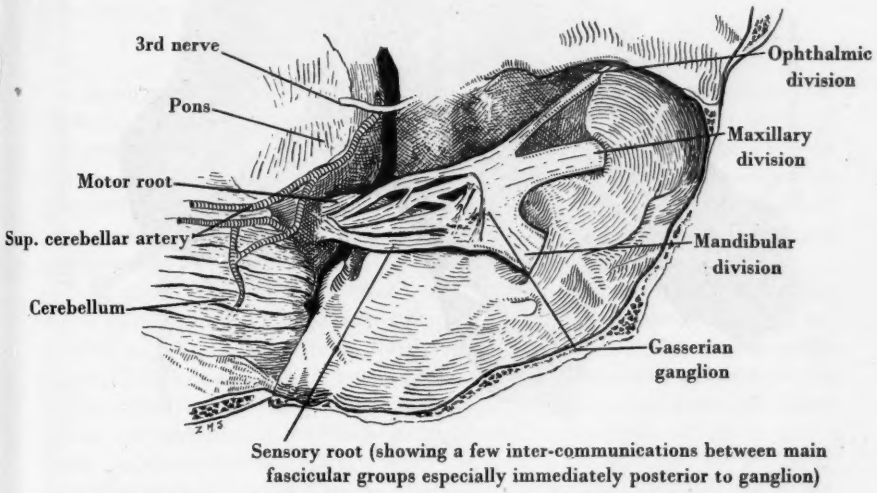

Fig. 9. Drawing of a dissection of semilunar ganglion and the central roots and peripheral branches of the trigeminal nerve. The rotation of the fibres in the central root can be clearly seen.

Fig. 10. Photograph of a dissection to demonstrate the arrangement of fasciculi within the central root of the trigeminal nerve.

with the superior portion of the ganglion and through this probably with the ophthalmic division, while the inferior portion of the root is similarly connected with the inferior portion of the ganglion and through it probably with the mandibular division of the nerve. The fibres of the intermediate portion of the root are less obviously connected to the middle portion of the ganglion owing to the seeming but false communications, but they mainly terminate in this manner.

(*b*) *The peripheral portion of the trigeminal nerve.*

The lingual nerve was chosen to represent the constitution of the peripheral portion of the fifth cranial nerve because of its large size and relatively long course, together with the absence of motor fibres and the presence of the chorda tympani. The latter being of different origin and function from the lingual it was of interest to discover whether or not this portion preserved its independence in the larger trunk.

It was found firstly that after its superficial union with the lingual nerve in the pterygoid region the chorda tympani simply continued forwards within the sheath of the lingual nerve as a quite separate fasciculus, until the point of origin of the terminal branches of the lingual nerve was reached. Here a coarse plexus existed and was obviously concerned with the distribution of the appropriate number of taste fibres to the various branches of the lingual nerve. Secondly, it was found that the lingual nerve from its origin to the point at which it commenced to branch consisted of parallel bundles of nerve fibres, there being no intraneural plexus formation. Some of these points are illustrated in fig. 11.

(4) *The facial nerve*

As an example of a non-segmental motor nerve the seventh was examined. Both the portions lying between the brain stem and the internal auditory meatus and that from the lower portion of the aqueduct to the anterior terminal branches were examined. Of the specimens examined in none was the portion of the nerve in the region of the geniculate ganglion in such a state of preservation as to allow of an adequate determination of its intraneural topography.

An examination of the central portion of the facial nerve, i.e. the rather short portion between the brain stem and the internal auditory meatus, revealed an absence of plexus formation—the piece of nerve being easily separable into parallel bundles of fasciculi. The facial nerve anterior to the stylomastoid foramen: as is well known and figured in the standard anatomical works the branches of the temporo-facial and cervico-facial divisions of the facial nerve communicate freely with one another in the face, the resulting complex leash of nerves forming the pes anserina. This arrangement is the equivalent of a coarse intraneural plexus, and as in cases in which such a plexus is formed there was no difficulty in separating the communicating

bundles from one another. They simply ran parallel to one another within a common sheath after the apparent union had taken place.

Within each of the main branches of the nerve there occurred at intervals intercommunications between the smaller bundles of fibres. These involved the smaller fibre groups, and in them the nerve bundles could not be separated as in the coarser plexuses.

In the course of the examination of the facial nerve another point of interest was noticed. As can be seen in the drawing of one of the dissections (fig. 12) three large communicating branches pass from the auriculo-temporal

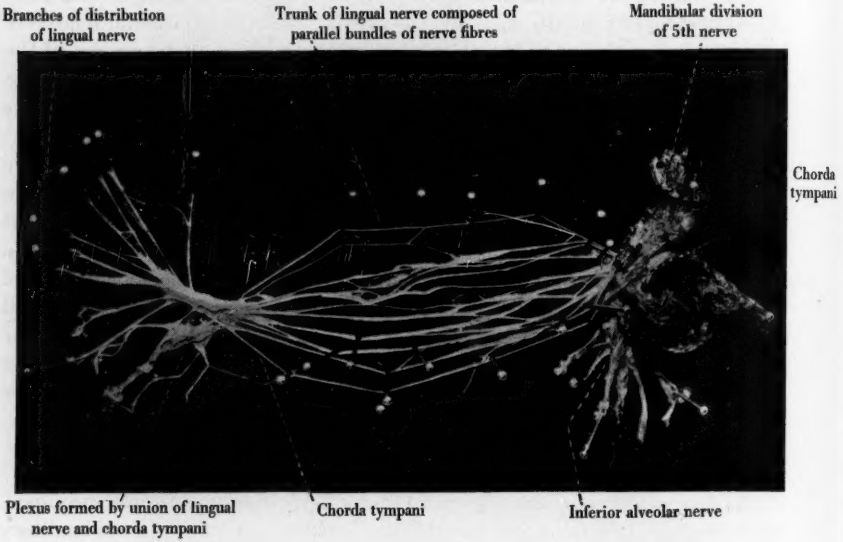
Branches of distribution of lingual nerve
Trunk of lingual nerve composed of parallel bundles of nerve fibres
Mandibular division of 5th nerve
Chorda tympani
Plexus formed by union of lingual nerve and chorda tympani
Chorda tympani
Inferior alveolar nerve

Fig. 11. Photograph of a dissection to demonstrate the fascicular arrangement within the lingual nerve. This also shows a distinct fasciculus which is the continuation forwards of the chorda tympani. This runs anteriorly within the lingual nerve sheath as far as the point of divergence of the peripheral branches of the lingual nerve.

nerve to the temporo-facial division of the facial nerve. On further dissection it was found that one of each of these communicating branches (two of them having previously divided) accompanied a branch of the cervico-facial division of the facial nerve to the periphery. In every case it was found that, while they appeared to form one nerve, the auriculo-temporal and facial fibres remained quite distinct from one another and simply proceeded forwards within a common nerve sheath. These communications from the auriculo-temporal nerve are known as the nerves of Frohse, and are concerned with the supply of fibres of the mandibular division of the trigeminal nerve to the skin of parts of the face and temporal region. The facial nerve simply conveys these groups of fibres to their peripheral distribution, and it is of interest to

note that the functional separation between the two groups is reflected in the anatomical arrangement.

Two other motor cranial nerves were examined—the hypoglossal and the spinal portion of the accessory nerves. In both the intraneural pattern was of a more complex type than might have been expected, and many anastomoses of finer fasciculi occurred throughout the trunks. This was especially well marked in the hypoglossal nerve beyond the point at which it received its communicating branch from the nerve loop between the first two cervical roots.

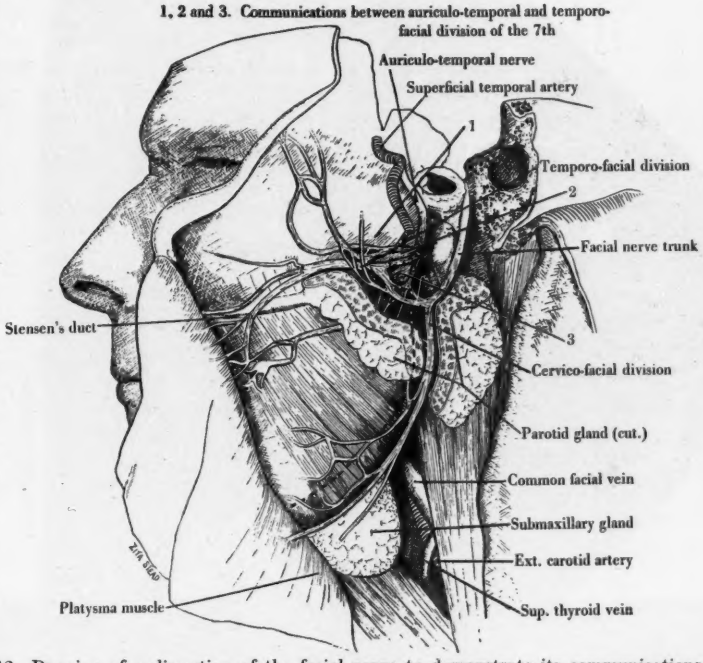

Fig. 12. Drawing of a dissection of the facial nerve to demonstrate its communications with the auriculo-temporal nerve (nerves of Frohse).

(5) *The plurisegmental nerves*

In the first place small nerves were examined. Of these both sensory and motor types were used. As an example of the former class the posterior cutaneous nerve of the thigh was investigated. It was found that communications occurred between fasciculi but that distal to the junction the two groups of fibres were easily separable, being still distinct from one another, though enclosed within a single sheath. In fig. 15 is seen the result of this dissection; the plexus has in great part been unravelled, but at one or two points the separation of fascicular groups has not been attempted so as to suggest the

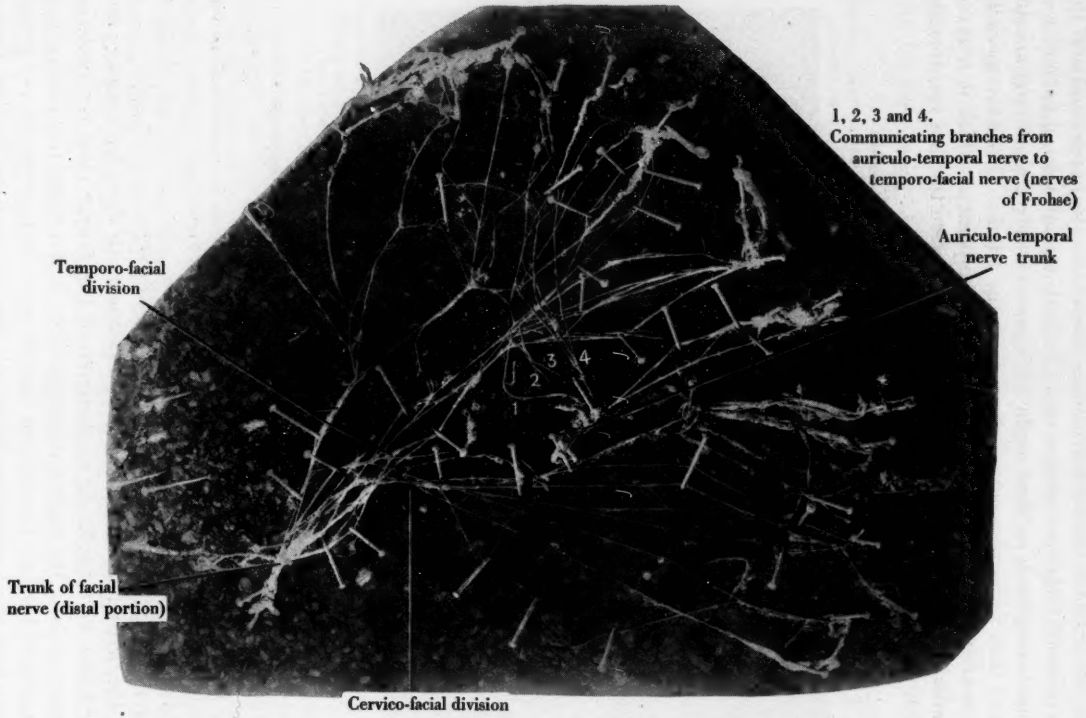

Fig. 13. Photograph of a dissection of the facial nerve to demonstrate fascicular pattern and the distinct bundles formed by the fibres of auriculo-temporal origin as they pass anteriorly within the branches of the facial nerve.

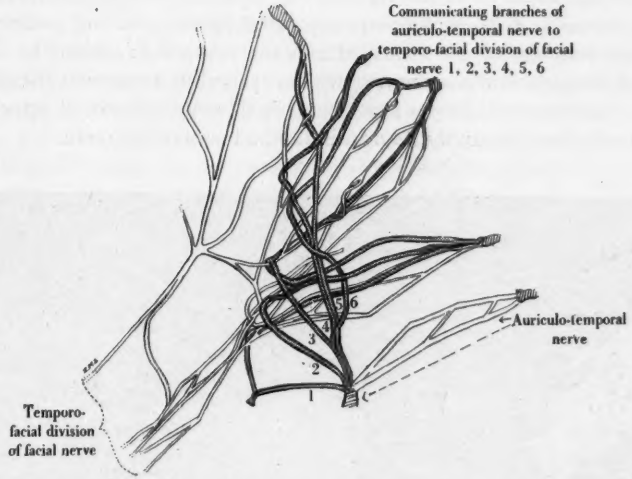

Fig. 14. Drawing of the area of the dissection shown in fig. 13 which contains the communicating branches of auriculo-temporal nerve to the cervico-facial nerve.

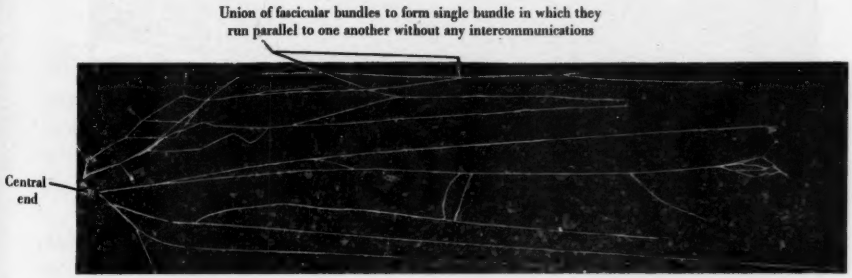
Union of fascicular bundles to form single bundle in which they run parallel to one another without any intercommunications
Central end

Fig. 15. Photograph of a dissection to demonstrate fascicular pattern in posterior cutaneous nerve of thigh. The dissection has not been quite completed as referred to in text.

original arrangement throughout the nerve. A dissection of some of the smaller fibre groups so obtained revealed an absence of plexus formation within them. The arrangement is therefore identical with that found in the lingual nerve with the exception of the coarse plexus above described. It is considered that from its nature the latter must be concerned with the grouping together of fasciculi from different segmental sources for the production of peripheral branches of the trunk. From the manner in which the fasciculi remained distinct from one another while enclosed in a common sheath, their apparent union can have no functional significance—it would appear that each is on its way to supply its own individual area of skin, etc.

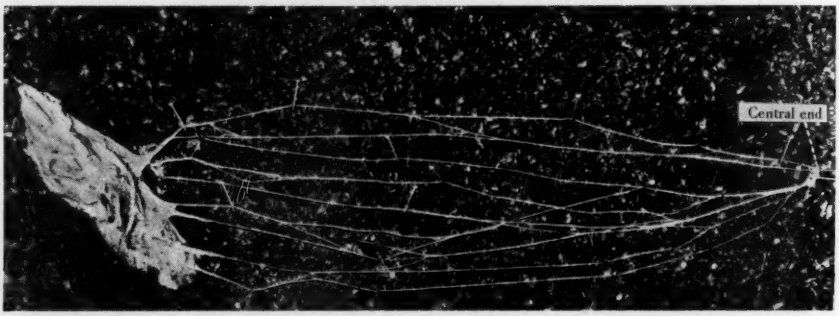

Fig. 16. Photograph of a dissection of the branch of the median nerve to the flexors of the wrist and fingers to demonstrate its intraneural topography.

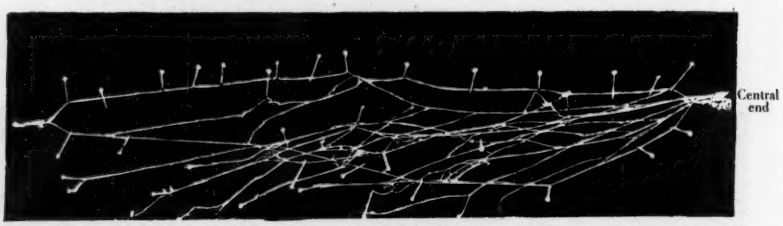

Fig. 17. Photograph of a dissection of the distal portion of the long thoracic nerve to demonstrate its intraneural topography.

Some small motor nerves in this group were next examined. The groups of branches of the median nerve to the flexors of the wrist and fingers were dissected first: the arrangement of the major groups of fibres is a simple one (fig. 16). Secondly, the distal portion of the long thoracic nerve was examined. In this relatively small trunk a finer dissection was made and a more complex intraneural pattern was discovered (fig. 17).

A brief examination of some of the larger plurisegmental nerves (median and ulnar) was carried out, and in each case a plexus of considerable complexity was seen, and, of course, this has been described by many workers. It is claimed here that if the intraneural plexuses in simpler nerves can be explained it is justifiable to suppose that those in larger nerves have a similar significance. In the course of the examination of these larger nerves it was of interest to find that in all the cases examined the true origin of a branch (the point at which its fascicular components had been collected together) differed from its apparent origin (the point at which it parted company with the main trunk). In other words the various branches could be stripped away from the main trunk for a varying distance without the division of any nerve fibres.

DISCUSSION

Before proceeding to consider these results the meaning which is attached to the expression intraneural plexus in this paper must be made clear. When describing the communications of the auriculo-temporal nerve with the seventh, or those between the fascicular groups in the posterior cutaneous nerve of the thigh, it was pointed out that following the union of two such groups of fibres these run together towards the periphery in a single sheath, apparently forming one fasciculus. However, if this sheath be incised, the two groups of fibres are found to have retained their individuality and are easily separable from one another. There is thus no intermingling of the fibres in the two groups and the union is more apparent than real.

On the other hand, an examination of the trunk of an intercostal nerve at its point of union with the grey ramus of the sympathetic shows the latter to terminate immediately in a splay of fibres—these being distributed throughout the intercostal nerve trunk. The grey ramus has lost its individuality; its fibres pass to the periphery in the various fasciculi of the larger nerve. In the same way within each of the main fibre groups into which the intercostal nerve may be split (fig. 4) there occur at intervals plexuses which involve an actual blending of the nerve fibres in two originally distinct groups. Beyond these plexuses separation of the fibre bundles was no longer possible by the methods employed in this work. While the difference between the coarse and the fine plexus may be simply due to the size of the nerve bundles, it is believed to be an important one.

A gradation in the type of plexus occurs as one passes from the centre towards the periphery—the large limb plexus and its branches, succeeded by a coarse fascicular plexus within these, and finally by a finer plexus of nerve fibres within these fasciculi. It is in the intimate blending that occurs at the last of these that one would expect the finest and functional regrouping of fibres to occur.

The results obtained from the examination of the various sensory nerves mentioned above will first be considered. In the optic nerve an absence of intraneural plexus formation has been shown, and it is suggested that this is

to be associated with its single function. Although other fibres than visual afferent ones do exist in the optic nerve it is believed that this does not detract from the general truth of the statement that this is a simple unifunctional nerve.

In the trigeminal nerve it has been shown that there is an absence of true plexus formation in the sensory root, that is, no intermingling of the smaller groups of nerve fibres occurs. There are, however, especially immediately posterior to the ganglion, a number of instances of the grouping together of fasciculi within a single sheath, as these are traced posteriorly to the pons. From its coarse nature it is believed that this has no functional significance and it might well be explained as follows. At the posterior margin of the semilunar ganglion the fibres of the sensory root are arranged in a relatively wide but thin band, corresponding in its dimensions to the posterior margin of the ganglion. At the pons, however, the fibres of the sensory root are compactly grouped into a mass oval in cross-section. In passing from the one arrangement to the other neighbouring fasciculi which were originally loosely packed become gathered together into a single sheath, this occurrence being most marked in the central region at the posterior margin of the ganglion. The fact that these fusions of fasciculi involve only groups of fibres which are already closely related must be remembered. It is of importance, since it implies that no gross disturbance of the relationships between fasciculi is brought about as a result of the fusions.

It must now again be stated that there is an absence of true plexus formation in the sensory root. In the same way it has been shown that there is no plexus within the lingual nerve—the largest of the purely sensory branches of the trigeminal. Unless there be some rearrangement of fibres within the ganglion itself, this implies that the nerve fibres in the central root are grouped in the same manner as in the peripheral portions of the nerve. In other words, in the central root there has been no separation of fibres to form functional groups (e.g. for pain or temperature fibres), but on the contrary a given area of cross-section of the central root corresponds to the sensory nerve supply of a certain area of the face or scalp and subserves all forms of sensation in that area. It has been previously mentioned that nothing can be said as to the arrangement of the fibres at their pontine attachment.

From the results of the dissections made in connection with this work it is considered that the ophthalmic, maxillary and mandibular divisions of the trigeminal nerve maintain their individuality in the sensory root. Such an opinion is in accord with the results of the majority of clinical observations in connection with the operation for trigeminal neuralgia and also with those of the experimental work on this subject. That the sensory loss following such partial sectioning is not always exactly what would be expected on anatomical grounds is not altogether surprising, nor does it detract from the general truth of the view on which the operation is based. One factor in the production of this irregularity would be the rotation of the root which has been seen to

occur. Of more importance would be the gross intercommunications above referred to. While these do not correspond to a true plexus and while they involve closely adjacent fasciculi only (therefore not disturbing the general topography of the root), they will account for the irregularities in the areas of anaesthesia following partial section which have been reported by some workers. It may here be mentioned that the results of partial section of the sensory root close to the pons following the subcerebellar approach cannot be explained on the findings here described.

It seems justifiable to suppose that what is found to be true in the largest of the sensory nerves will be true of other sensory nerves as well. That is, that the afferent fibres in a posterior nerve root are grouped together in terms of their peripheral distribution—that the grouping here is a topographical and not a functional one. One can therefore state that any plexus formation found in a peripheral nerve cannot be concerned with the grouping together of different types of afferent fibres, since these are already correctly grouped in the posterior roots in respect of their peripheral distribution.

Before proceeding to consider nerves of other function the topography of the last of the pure sensory nerves examined must be described. As has been mentioned above, a coarse type of plexus existed within the posterior cutaneous nerve of the thigh. No intermingling of the constituent fibres in communicating fasciculi was seen however, and it is considered that the plexus was due to the grouping together of fibres from various segments for the formation of various branches. Since the sensory fibres from each segment are distributed to their own particular area of skin there is no occasion for the intermingling of the segmental groups, although these are in intimate contact within a common sheath. In concluding this section it may therefore be stated that true plexus formation does not occur in pure sensory nerves.

Of the pure non-segmental motor nerves the facial was most carefully examined. It has been pointed out that in a short length of nerve between the brain stem and the internal auditory meatus no plexus formation was observed. In the facial nerve peripheral to the stylomastoid foramen intercommunications between fasciculi occurred at two points: firstly, those passing between the main branches of the nerve (forming the pes anserina), and secondly, within each of the branches of the nerve there were at intervals intercommunications between the smaller fascicular groups. The first of these plexuses was of coarse type and the communicating fasciculi were easily separable. It is suggested that these result not from any functional need but from the complexity of the facial muscular sheet. The second is an example of true intraneural plexus formation, involving the regrouping of the smaller fasciculi, and it is here suggested that it can best be explained as being the result of the grouping together of muscle afferent and efferent fibres. It is of interest to note that the fibres of the auriculo-temporal nerve, having no functional relationship to those of the seventh, remain quite distinct from them. Although both groups of fibres run for considerable distances within

the same sheath, no intercommunications occur between them. It suggests immediately that mere close relationship does not give rise to an intraneural plexus—there must be some other reason for this occurrence.

Two other non-segmental motor nerves were examined—the hypoglossal nerve and the spinal portion of the spinal accessory nerve. It was found that the arrangement on the whole was more complex than was expected. The fine intraneural plexuses existing within the large fascicular groups is likely to be again concerned with the grouping together of efferent and afferent muscular fibres. No other function can be suggested.

In the smaller plurisegmental motor nerves, as has been seen, a plexus is found in each of those examined. In its coarser part this was probably similar to that found in the posterior cutaneous nerve of the thigh, but the finer true plexus, e.g. that in the distal part of the long thoracic nerve, corresponds to that seen in the other motor nerves mentioned above, and it is here suggested that it is of like significance. An intraneural plexus is therefore found in all motor nerves. In its coarser forms it probably depends on the origin and subsequent developmental history of the muscle, while the finer plexus may well be the result of the grouping together of muscle efferent and afferent fibres.

The findings in connection with the intercostal nerves may next be considered. While unisegmental these are mixed nerves from the functional standpoint, and a careful investigation of their topography was made as is described above. The absence of plexus formation in the anterior and posterior roots of the spinal nerves has been noted—that in the latter being what would be expected on analogy with the trigeminal nerve. It is more obvious, however, in that not even the gross intercommunications noticed in the central root of the semilunar ganglion are to be found. Following the union of the two roots a plexus develops which extends forwards in the nerve trunk for some distance. Its first function would appear to be the grouping together of fibres from the two roots into anterior and posterior primary divisions. Further grouping of these fibres, however, occurs here to produce the large fasciculi which have been seen to exist in the nerve trunk anterior to the plexus. It will be remembered that distal to the plexus these fasciculi are easily separable from one another. A final complicating factor in the region under consideration is the entry of post-ganglionic sympathetic fibres into the nerve trunk and the passage of some of these fibres to each of the main fascicular groups above referred to, before these separate from one another.

It has been pointed out that within the main fascicular groups there occur at intervals intercommunications which involve a reassortment of the smaller groups of fibres. It is here suggested that this plexus is concerned with the associating together of muscle afferent and efferent fibres. In other words it is believed that this plexus has a function similar to that described in pure motor nerves. Of course, it is possible that the association of muscle afferent and efferent fibres commences in the coarse plexus which exists immediately beyond the union of anterior and posterior nerve roots. But this association

is an intimate one and would best be secured by the intermingling of the smallest fibre groups.

This investigation was undertaken in an endeavour to determine the significance of the intraneural plexus—especially of the complex plexuses to be found in the large nerve trunks of the limbs. An endeavour will now be made to explain this plexus by considering it in the light of the results of the examination of simpler nerves. In addition to the groupings of fibres which occur in an intercostal nerve, there is in a plurisegmental nerve the added necessity of grouping together fibres from the different segments within the trunk for the production of the various plurisegmental branches. It is here suggested that in such a nerve trunk plexus formation is due to a variety of causes. In its proximal part there occurs a grouping together of afferent and efferent fibres similar to that seen in intercostal nerves. Then following the union of this trunk with a trunk or trunks from other segments there occurs the complex series of intercommunications resulting in the production of the requisite segmental groupings for the collateral intraneural branches. Then within the larger fibre groups occur finer plexuses of smaller fibre groups, which it is thought may be concerned with the association of muscle afferent and efferent fibres.

Practical significance of results

The relationship of the question of intraneural plexus formation to the rationale of partial section of the sensory root of the fifth nerve for trigeminal neuralgia has been already discussed at some length. The absence of true plexus formation in the central root has been noted and an explanation suggested for such communications between fasciculi as do occur. It has, therefore, been stated that unless some rearrangement of fibres occurs within the ganglion the various portions of the central root must correspond to the peripheral division of the nerve (i.e. the upper and inner part of the root to the ophthalmic division, a larger number of fibres in its lower and outer part to the mandibular division, while the maxillary division would be represented by an area intermediate in size and position). The ganglion itself must be similarly divisible into three portions. The rotation of the root which occurs as this approaches the pons must be borne in mind, for, to obtain the same area of anaesthesia, the site of a partial section must be varied according to the point at which the root is divided—especially if this be close to the pons.

The subject of intraneural topography has its interest also in connection with the well-known statement of Sherren that one-third of the sectional area of a nerve trunk may be divided without the production of any demonstrable anaesthesia or sensory loss. This statement has been criticised and described as untrue, but at least in the large plurisegmental limb nerves it is not only theoretically possible but indeed likely.

Owing to the reassortment of fibres in progress in the proximal portion of such a nerve trunk a partial section of any one point does not abolish the

function of any one particular branch to the skin or to muscle, but instead destroys a fraction only of the fibres proceeding to their distribution through several branches. Such a reduction in the number of fibres in several branches is unlikely to show itself as a paresis or sensory loss (unless the original section has been of greater extent than one-third).

Sherren also pointed out that if the section be made immediately proximal to one of the branches of the trunk the structures supplied by that branch will always be denervated. This again is in accordance with the findings here set out, for each peripheral branch has collected together its various functional and segmental components from the main nerve trunk at a variable point above the actual superficial origin of the branch. In other words, the true origin of a branch is often quite different from its superficial origin, and it is obvious that a partial section of the trunk between these points and on the side of the nerve from which it takes origin will be equivalent to a division of the branch itself.

This latter point is also of interest in connection with Stoffel's operation of motor neurectomy. The results described in this paper are opposed to the tract localisation in peripheral nerves described by Stoffel. However, as has just been mentioned, the true and apparent origins of branches may be separated by some distance, e.g. branches of median nerve to flexors of wrist and fingers, and here the operation as practised by Stoffel is in accord with anatomical findings. In the case of obturator neurectomy the nerve being an almost pure motor nerve can be justifiably partially divided, but here the branches of the nerve to the various adductors are often separately sectioned.

The problem was at first thought to have some interest in connection with certain of the results of nerve injuries. In some partial nerve injuries the clinical picture is one of a distal palsy, together with anaesthesia of variable extent—the muscles in the proximal portion of the limb being quite unaffected. Since no record of this condition being the *immediate* result of an injury can be obtained, it is considered that it is best explained by supposing that regeneration has not affected the distal muscles (temporarily or permanently).

SUMMARY

1. The problem of the significance of intraneural plexus formation has been investigated by the dissection of various nerves, some functionally simple and others with several functions and derived from several spinal segments.

2. The absence of intraneural plexus formation in certain nerves has been demonstrated. As examples may be mentioned the optic nerve, the anterior and posterior roots of the spinal nerves, and the sensory and motor roots of the trigeminal nerve.

3. The absence of intraneural plexus formation in the sensory root of the fifth nerve and in the largest of its purely sensory branches—the lingual nerve—has been described. This suggests that the grouping of the fibres in the sensory root repeats that in the peripheral branches. It is further sug-

gested that the same is true of the sensory fibres in other nerves, so that such plexus formation as occurs in them cannot be due to the regrouping of afferent fibres.

4. Intraneural plexus formation has been shown to occur in all the peripheral motor nerves. While it is believed that the coarser interfascicular communications may be related to the embryological history of the muscle innervated, it is considered that the finer plexus serves to group together muscle afferent and efferent fibres.

5. It has been shown that intraneural plexus formation of two types occurs in unisegmental nerves. In the first place, immediately beyond the union of the anterior and posterior nerve roots there is a coarse plexus, which appears to be concerned with the grouping of fibres from the anterior and posterior roots into the relatively large parallel fasciculi to be found anterior to the plexus. This plexus is complicated by the entry into the nerve at this point of the grey sympathetic ramus. In the second place, within the fasciculi thus produced intercommunications between smaller fibre groups occur at intervals. These involve the intermingling of the fibres within the smaller fasciculi, and are believed to be concerned in the association of muscle afferent and efferent fibres.

6. In the larger plurisegmental nerves it is suggested that intraneural plexus formation is due to a combination of the factors mentioned above.

At the root of the limb there occurs the grouping together of afferent and efferent fibres into unit groups and the distribution of sympathetic fibres into these groups. Then follows the union of this nerve trunk with other trunks from neighbouring segments at the main limb plexus (e.g. brachial). Within the enveloping sheath there now occurs the gradual grouping together of fibres from various segments with the production of the collateral and terminal branches. Finally, within the larger groups of fibres there takes place an interlacement of the smallest bundles of fibres, and it is here that one would expect the functional association of muscle afferent and efferent fibres to occur—for this demands an association of the smallest groups of fibres. Thus as one passes from the centre towards the periphery one encounters first coarse plexus formation with the production of a coarse grouping of fibres. Then within these groups there occur further and finer plexuses which are to produce the final arrangement of fibres as they approach their areas of distribution.

7. The practical significance of the problem is discussed in relation to the surgical treatment of trigeminal neuralgia and to certain results of partial nerve division which it is thought can be explained satisfactorily on the findings described above. Reference is also made to the connection between the modern views on intraneural topography and Stoffel's operation of motor neurectomy.

In conclusion I have very great pleasure in recording my indebtedness to Prof. Woollard. The facilities offered to those working in his department are

indeed excellent, but it is for his advice and never-failing encouragement that I am most grateful, and without these the work would never have been completed.

I must also express my thanks to Mr H. Platt, who advised me on some of the clinical points, and finally to Miss Stead for her careful drawings and her assistance in preparing and labelling the photographs.

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WOUND HEALING *IN VITRO*

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INTRODUCTION

It is a well-known fact that, in the healing of a wound in the animal body, when the epithelium has grown over the proliferating connective tissue, growth of the connective tissue stops; a state of equilibrium is established in which the active fibroblastic multiplication and diffuse fibril formation cease and the tissue assumes the orderly arrangement of a quiescent scar.

The physiological basis of this tissue interaction is part of the general problem of tissue balance. It has been shown experimentally that in early embryonic development certain tissues induce the development of the adjacent tissues. This process is not confined to embryonic cells, as demonstrated by Huggins (1931), who showed that portions of bladder mucosa transplanted into the abdominal wall of the dog or rabbit induce the formation of bone in the adjacent connective tissue. In teratomata also (Willis, 1935) certain epithelial glandular elements appear to determine the arrangement and differentiation of the surrounding mesenchyme cells.

Whether an analogous principle applies to the tissue interactions of wound healing is not known, and a histological study of wound healing *in vivo* affords no clue to the physiological processes involved. It was therefore decided to approach the problem of the interaction of dermis and epidermis in cutaneous wound repair by experiments *in vitro*, which would enable the behaviour of the two tissues to be studied free from extraneous influences of the body. By cultivating epidermis in contact with dermis any changes produced in the connective tissue under the influence of the growing epithelium would be detected, and if such changes appeared it might be possible to investigate their physiological basis.

MATERIAL

The skin was obtained from full-term rat embryos, which were removed aseptically by abdominal hysterotomy a few hours before birth. At this stage the skin is almost fully differentiated, except that the growth of the hair follicles and glands is still incomplete. It was impossible to use post-embryonic skin as strict asepsis was essential.

TISSUE CULTURE

All explants were grown by the watch-glass method (Fell and Robison, 1929). In this method the culture vessel is a watch-glass enclosed in a Petri dish carpeted with a layer of wet cotton-wool. The watch-glass contains a clot

composed of four drops of fowl embryo extract and four drops of fowl blood plasma. The extract was made with Pannett and Compton's saline from the minced tissue of a 10–11 day embryo.

In no case was an explant grown for more than 3 days, so that transference of the explant to fresh clot in another culture vessel was not required.

HISTOLOGY

All material was fixed *in situ*, in the culture vessel, in Carnoy's fluid. After fixation the explant, with a margin of clot, was cut out, dehydrated, cleared in methyl benzoate containing 1 per cent. celloidin and embedded in paraffin wax. The serial sections were stained with Heidenhein's modification of Mallory—the Azan stain.

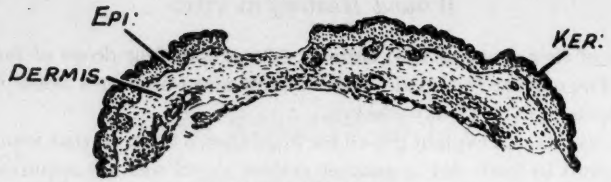
WOUND HEALING IN SKIN CULTURES

In these experiments a minute piece of tissue was excised from the centre of a small square of skin, producing a circular wound extending into the dermis (text-fig. 1). The skin was then explanted *in vitro*. Thirty such explants were made and were fixed at different periods of cultivation for histological examination.

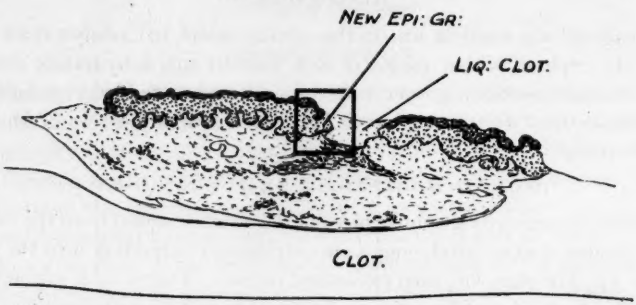
Epithelium and connective tissue grow into the wound which heals in 2–3 days. The different stages in this process can be followed in the histological preparations.

An explant fixed after 18 hours *in vitro* (text-fig. 2 and Plate I, fig. 1) shows the epithelium beginning to spread over the defective area, whilst the fibroblasts round the edge of the wound have multiplied and the intercellular fibrils have become thicker and more densely arranged; both fibroblasts and fibrils tend to turn up into the wound beneath the growing epithelial edge. After 36 hours' cultivation these changes are more marked (text-fig. 3 and Plate I, fig. 2), and at the margins of the wound a dense mass of collagen fibrils is sweeping up towards the proliferating epithelium. This appearance stops abruptly at the border of the ingrowing epidermis, and little change is seen in the dermis in the interior of the wound which the epithelium has not yet reached. As the epithelium spreads over the defect, this peculiarly dense, orientated arrangement of the dermal fibrils also extends towards the centre in exact relation to the epidermal growth, so that when the epidermal edges finally fuse the entire dermis of the wound has assumed this altered structure.

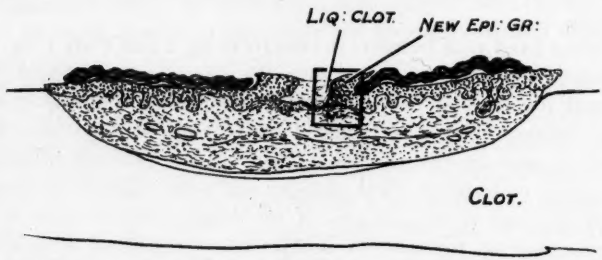
When the wound has healed and epidermal proliferation has ceased the dermis reverts to its original structure. The fibrils gradually lose their orientation to the epithelium, fibroblastic activity stops, and the dermis now consists of rather dense, irregularly arranged fibrils among which lie a few inactive fibroblasts. The orientation of the fibrils first disappears at the margin of the wound (text-fig. 4 and Plate I, fig. 3), and it is significant that this is the region where the basement membrane of the epidermis has reformed; near the centre of the wound, where as yet there is no basement membrane and the recently



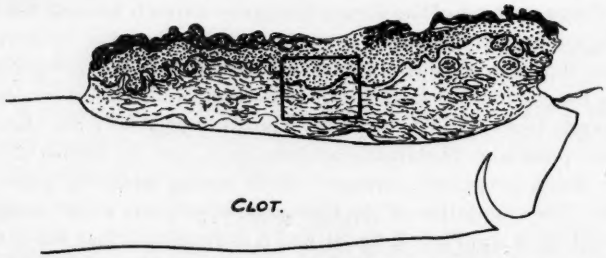
Text-fig. 1. Section of explant of skin, showing central wound. *Ker.* = keratin layer. *Epi.* = epidermis. (× 50.)



Text-fig. 2. Section of explant fixed after 18 hours *in vitro*, showing wound healing. *Liq.clot.* = liquified clot filling central wound. *New epi.gr.* = epithelial new growth. The area enclosed by the black square is seen under high magnification in Plate I, fig. 1. (× 40.)



Text-fig. 3. Section of explant fixed after 36 hours *in vitro*, showing wound healing. Abbreviations as in text-fig. 2. Note extent of epithelial new growth. The area enclosed by the black square is seen under high magnification in Plate I, fig. 2. (× 40.)



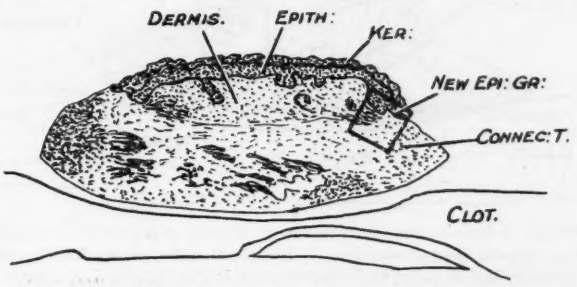
Text-fig. 4. Section of explant fixed after 54 hours *in vitro*, showing healed wound. The area enclosed by the black square is seen under high magnification in Plate I, fig. 3. (× 56.)

active epithelium is still in direct contact with the connective tissue, the orientation of the fibroblasts and fibres persists. Eventually a basement membrane is formed over the whole wound and the changed structure of the dermis disappears even in the interior.

The above results show clearly (*a*) that during the healing of a wound *in vitro* marked changes occur in the dermis, viz. an active cell proliferation associated with an increased density and orientation of the inter-cellular fibrils towards the epidermis, (*b*) that these changes appear only beneath the ingrowing epidermis, and (*c*) that this new structure of the dermis is lost when epithelial regeneration ceases and the basement membrane reforms.

GRAFTING EXPERIMENTS

The following experiments were made in order to investigate more fully the interaction between the epidermis and connective tissue.



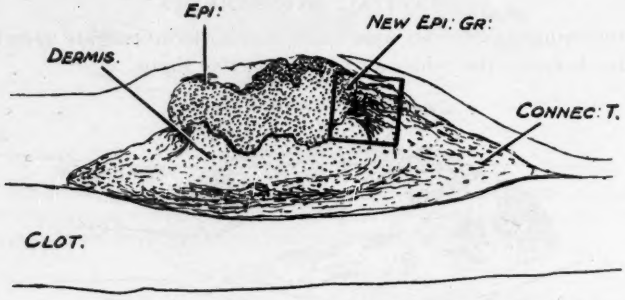
Text-fig. 5. Section of explant after 48 hours *in vitro*, showing skin grafted on to a layer of connective tissue. *Epith.* = epidermis of graft. *Dermis* = dermis of graft. *Connec. T.* = connective tissue on which skin graft is laid. *New epi. gr.* = epithelium growing out from graft over the connective tissue. The area enclosed by the black square is seen under high magnification in Plate I, fig. 4. (×40.)

The layer of loose connective tissue adherent to the under-surface of the skin was scraped away and portions were explanted on the surface of the clot. A small square of embryonic skin was then laid on each explant and the culture incubated; in effect a piece of entire skin was grafted on to a layer of connective tissue. Twenty-four of these experiments were made and the explants were fixed and sectioned at different periods of growth.

The epithelium of the skin grows over the underlying connective tissue. Sections of an explant fixed after 48 hours' growth (text-fig. 5) show the epidermis extending a short distance over the fibrous tissue beyond the limits of the original graft. The connective tissue immediately beneath the growing epithelium has begun to change its character (Plate I, fig. 4). Some of the fibroblasts are in a very active state, with enlarged cytoplasm and a large nucleus containing very prominent chromatin granules. The first indication of an orientation of the fibroblasts towards the epithelium is seen, but as yet there is no alteration in the density or arrangement of the intercellular fibrils.

After 3 days' cultivation (text-fig. 6 and Plate II, fig. 1) the reaction of the connective tissue is more advanced. There is intense fibroblastic activity in the neighbourhood of the growing epithelial edge; there the fibroblasts are much more numerous than in more remote regions, many mitotic figures occur and the cells are orientated at right angles towards the epithelium. The intercellular fibrils have also multiplied greatly to form a comparatively dense mass, and are orientated parallel with the fibroblasts.

It is thus clear that, under the conditions of the experiment, epidermal epithelium can influence profoundly the activity, rate of growth and arrangement of connective tissue growing in contact.



Text-fig. 6. Section of explant after 72 hours *in vitro*, showing skin grafted on to a layer of connective tissue. Human embryo material. Abbreviations as in text-fig. 5. The area enclosed by the black square is seen under high magnification in Plate II, fig. 1. (×56.)

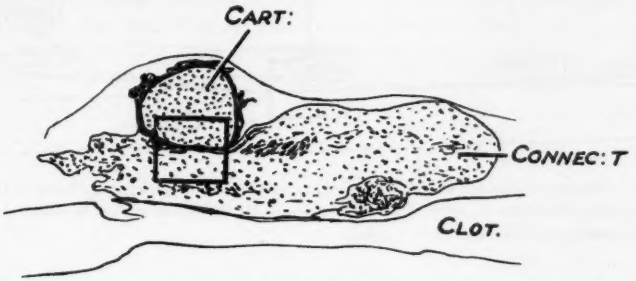
THE EFFECT OF A DIFFUSION CURRENT ON EXPLANTED CONNECTIVE TISSUE

The effect of epidermal epithelium on the growth of connective tissue *in vitro*, though very striking, differs markedly from what occurs during wound healing *in vivo*. It is therefore necessary to consider whether the effects observed could be produced artificially by the conditions of the experiment.

Weiss (1933) showed that a diffusion gradient could orientate the colloidal particles of the ground substance of a tissue in the direction of flow, and that cell migration tended to follow the lines of orientation. Since cellular multiplication depends upon the supply and circulation of fluid, growth is also facilitated along these lines.

It seems reasonable to suppose that the rapidly growing epithelial margin, requiring salts and water, must absorb them from the subjacent connective tissue, which in turn absorbs them from the underlying clot. In this way diffusion currents might pass from the clot through the connective tissue and converge on the epithelial edge of the wound. On this hypothesis the orientation and active new growth in the connective tissue would occur along the lines of the current.

It is difficult to believe, however, that the epithelial margin could absorb enough fluid to establish a current in the connective tissue strong enough to cause the marked activation; but, nevertheless, the hypothesis was tested experimentally in the following way. A piece of embryonic cartilage was dehydrated in acetone and the acetone evaporated from the cartilage in a desiccator. The dry shrivelled cartilage was then placed on an explant of connective tissue and the culture incubated. The dried cartilage thus took the place of the skin graft in the previous experiments. The cartilage is hygroscopic and for 1–2 days gradually absorbed water from the culture, so that a diffusion current must have passed at right angles through the connective tissue, since the ultimate source of the fluid was the clot. Six such experiments were made and the explants were fixed and sectioned.



Text-fig. 7. Section of explant showing cartilage in contact with connective tissue, after 36 hours *in vitro*. The area enclosed by the black square is shown under high magnification in Plate II, fig. 2. (×40.)

The histological preparations showed no trace of an orientation of the collagen fibrils or fibroblasts towards the cartilage, but rather a capsular arrangement around the cartilage (text-fig. 7 and Plate II, fig. 2). The influence of diffusion currents in the production of the lines of growth in the dermis can therefore be discounted.

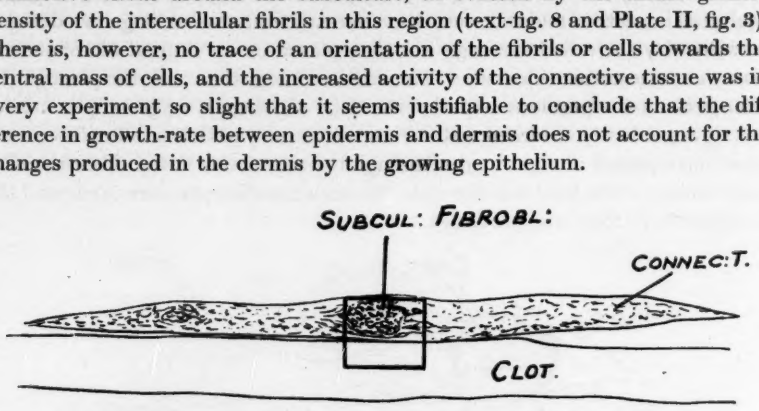
THE EFFECT OF ACTIVELY PROLIFERATING CELLS ON CONNECTIVE TISSUE

In skin cultures the dermis grows much more slowly than the epidermis. It therefore seemed possible that the more rapidly growing epithelium, by secreting digestive ferments into the available food substances in its neighbourhood, might provide a nutritive digest for the underlying connective tissue, causing a local stimulation of dermal growth immediately beneath the growing edge of the epithelium. If this were so, the changes observed in the dermis during wound repair *in vitro* would not be a specific effect of the epidermal growth.

In order to test this possible explanation six experiments were made, in which an actively proliferating subculture of fibroblasts was placed on the surface of a layer of connective tissue *in vitro*. The fibroblastic cultures were

usually taken to the fourteenth passage before being laid on the connective tissue.

Sections of such preparations show a slight increase in the activity of the connective tissue around the subculture, as evinced by the rather greater density of the intercellular fibrils in this region (text-fig. 8 and Plate II, fig. 3). There is, however, no trace of an orientation of the fibrils or cells towards the central mass of cells, and the increased activity of the connective tissue was in every experiment so slight that it seems justifiable to conclude that the difference in growth-rate between epidermis and dermis does not account for the changes produced in the dermis by the growing epithelium.



Text-fig. 8. Section of explant showing subculture of fibroblasts growing on layer of connective tissue, after 48 hours *in vitro*. The area enclosed by the black square is shown under high magnification in Plate II, fig. 3. (×40.)

THE EFFECT ON CONNECTIVE TISSUE OF EPITHELIA OTHER THAN EPIDERMIS

These experiments were undertaken with the object of finding whether other types of growing epithelium could produce the same effect on connective tissue as proliferating epidermis.

Fifteen experiments were made in which other types of epithelium, particularly submaxillary gland and kidney, were grafted on the explants of connective tissue. The epithelium grows out over the connective tissue in a similar way to the epithelium of the skin, but in no case was any effect observed in the connective tissue (Plate II, fig. 4).

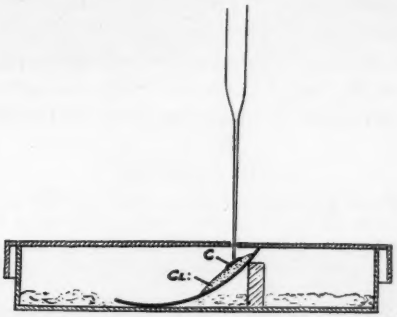
From these results it seems clear that the effects produced in connective tissue by growing epidermal epithelium are specific to epidermis.

THE BASIS OF THE CONNECTIVE TISSUE REACTION

The epidermis may produce the reaction in the dermis by mechanical, physical or chemical means. There is presumptive evidence from Huggins' experiments with bladder mucosa and definite evidence in the organisation of tissues in the early embryo, that the organising effects are due to a chemical substance formed in the organiser, which diffuses into the neighbouring cells and induces in them their new activity.

For this reason it was decided to investigate first the possibility of a chemical basis for this interaction between epidermis and connective tissue *in vitro*. Preliminary experiments have therefore been made in an attempt to determine whether the changes in the fibrous tissue are caused by some chemical substance formed by the growing epithelial cells.

The effect of two fluids, possible containers of an activating chemical substance, have so far been investigated. As skin epithelium grows out from the original explant, it liquefies the clot on which it lies so that the tissue is soon bathed in a small drop of liquid; this drop, which is in intimate contact with the growing cells, was collected from twenty to thirty cultures of skin and was the first fluid tried. The second was an extract of newly growing epithelium from a similar number of cultures; the sheets of epithelium were stripped from the clot and collected in a tube; the cell structure was broken down by intense freezing and the tissue was then crushed and extracted with saline.



Text-fig. 9. Diagram of culture-perfusion experiment. *C.* = explant of connective tissue. *Cl.* = clot.

By means of a fine glass pipette drawn to a microscopic point it is possible to deliver the fluid to a point close to the margin of a culture of fibrous tissue, and by suitably controlling the angle of the culture vessel and the air entry into the pipette the fluid can be made to diffuse slowly through the lower part of the explant for a period of 24 hours (text-fig. 9). Histological sections of the cultures after such experiments show that neither fluid has produced changes in the tissue comparable with those produced by growing epidermal epithelium. Whether this result means that the basis of the interaction between epidermis and dermis is not chemical, or that the active chemical substance, if present, requires different methods of isolation, concentration or presentation to the connective tissue culture, cannot yet be decided and further investigation is required.

SUMMARY

1. In cultures *in vitro* of embryonic rat skin epithelial growth influences profoundly the activity, rate of growth and arrangement of the dermal connective tissue, finally resulting in the formation of a dense mass of collagen fibrils orientated at right angles to the epithelium.

2. These changes in the connective tissue depend upon the presence of actively growing epidermal cells, and the new structure is lost when the basement membrane reappears beneath the new epithelium.

3. The reaction is not due to local conditions inherent in the experiment.

4. The reaction appears to be specific to epidermis and is not produced in response to other growing epithelia.

5. Preliminary experiments are described, undertaken in order to ascertain if the action of epidermal epithelium on connective tissue has a chemical basis.

It is a pleasure to express my gratitude to Dr H. B. Fell and Dr Jacobson, of Strangeways Laboratory, Cambridge, for their help, criticism and advice, and to acknowledge my appreciation of the services of Mr S. P. Steward and the technical staff of the Royal College of Surgeons Laboratories. The early stages of this work were assisted by a grant from the Dickinson Trust, University of Manchester.

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EXPLANATION OF PLATES I AND II

Plate I

Fig. 1. Wound margin of explant fixed after 18 hours *in vitro*—see text and text-fig. 2. Note thickened fibrils turning up at wound margin. (×500.)

Fig. 2. Wound margin of explant fixed after 36 hours *in vitro*—see text and text-fig. 3. Note thickened orientated arrangement of fibrils in relation to epidermal new growth (*A*). (×500.)

Fig. 3. Recently healed wound after 54 hours *in vitro*—see text and text-fig. 4. Note loss of thickened orientated arrangement of fibrils in region where basement membrane of epidermis has reformed. (×500.)

Fig. 4. Connective tissue beneath proliferating epidermis after 48 hours *in vitro*—see text and text-fig. 5. Note streaming of cells towards epithelium. (×500.)

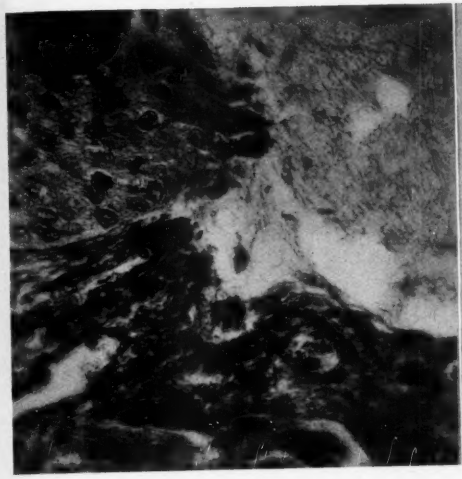
Plate II

Fig. 1. Connective tissue beneath proliferating epidermis after 72 hours *in vitro*—see text and text-fig. 6. Note intense fibroblastic activity and presence of thick mass of fibrils orientated towards epidermis. (×500.)

Fig. 2. Area of contact between cartilage and connective tissue—see text and text-fig. 7. Note capsular arrangement of fibrils and cells. (×500.)

Fig. 3. Fibroblastic subculture (*A*) growing on layer of connective tissue (*B*) after 36 hours *in vitro*. Note fibrils are not thickened and are arranged in capsular manner beneath the subculture. (×500.)

Fig. 4. Section of explant showing kidney epithelium growing in contact with connective tissue. *K*=kidney explant. *R*=kidney epithelium growing over the connective tissue (*C*). Note fibrils are not thickened and are arranged irregularly. (×500.)



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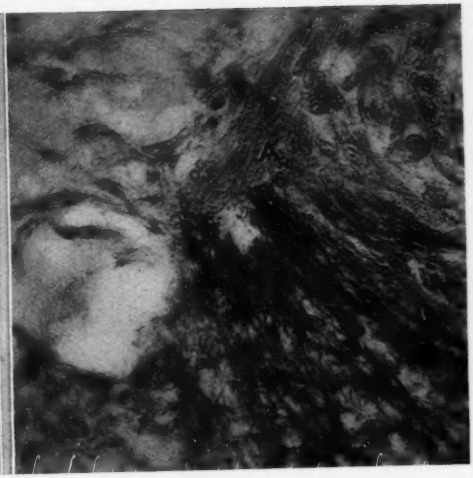

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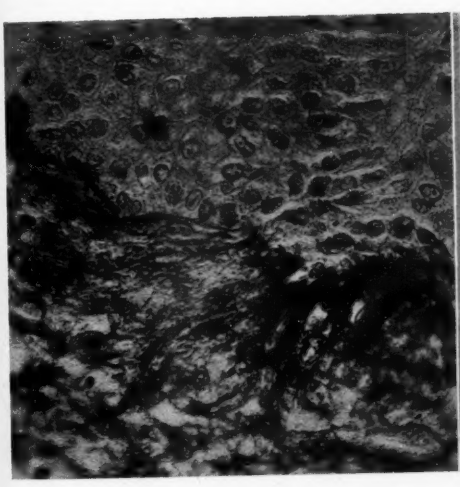

Fig. 3.

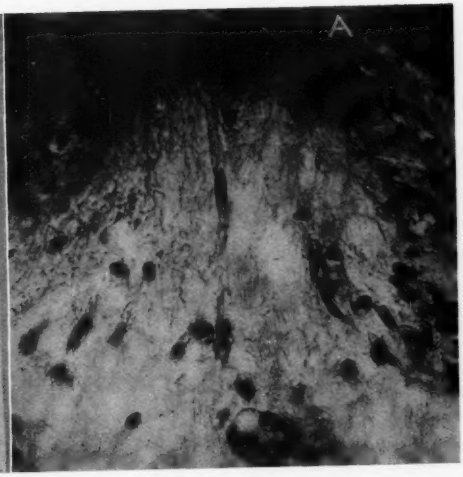

Fig. 4.

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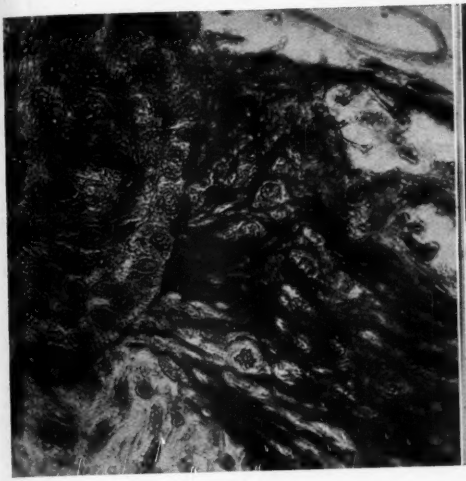

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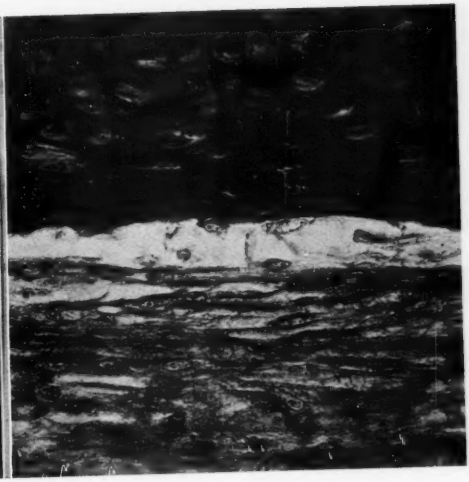

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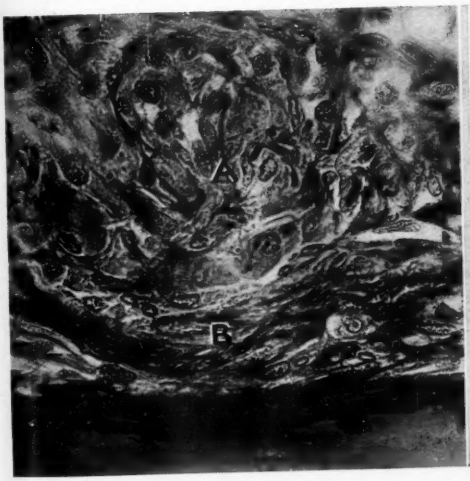

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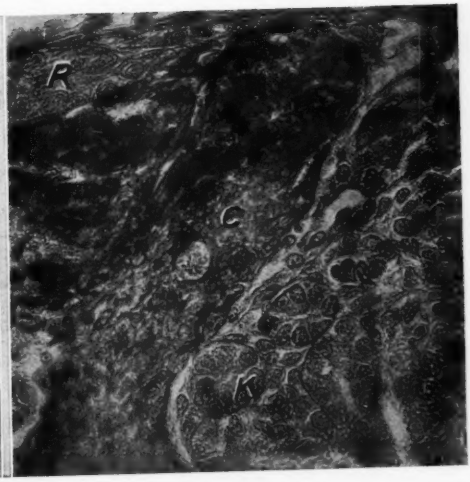

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VARIATION IN LYMPHOCYTE PRODUCTION

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INTRODUCTION

It has been noted, during the quantitative study of lymphocyte production, that the number of lymphocytes to be found in the thoracic duct lymph of the dog may show considerable variation. In previous work the fact that this variation may occur was not sufficiently appreciated (Yoffey, 1933). Many of the earlier experiments happened to be on animals in which the lymphocyte count remained fairly constant, thus according with the findings of Rous (1908). But even among these earlier experiments there were a few which showed marked variation. In two experiments, for example, of which details have already been given (Yoffey, 1933), one shows a series of hourly counts which undergo very little change (Table I), whereas the other shows a progressive fall in the number of lymphocytes (Table II).

Table I

Dog. 22. iii. 32

Time	Lymphocytes per c.mm. of lymph	Lymph c.c. per hour	
1.25 p.m.	11,500	25·0	(collection begun at 12.25 p.m.)
2.25 p.m.	11,300	23·0	
3.25 p.m.	12,200	22·0	
4.25 p.m.	11,100	26·0	
5.25 p.m.	11,300	23·0	
6.25 p.m.	10,000	23·0	

Table II

Dog. 26. iv. 32

Time	Lymphocytes per c.mm. of lymph	Lymph c.c. per hour	
3.12 p.m.	33,400	23·0	(collection begun at 2.12 p.m.)
4.12 p.m.	14,200	23·0	
5.12 p.m.	13,300	23·0	
6.12 p.m.	12,100	22·0	

The observation of such changes in lymphocyte count occurring during the course of a single experiment suggested the possibility of variations occurring as between one animal and another, and led to a comparison of lymphocyte production in different animals. It is clear that merely to compare lymphocyte counts per c.mm. of lymph is insufficient. What is required is a knowledge of the total lymphocyte output, i.e.

(lymphocyte concentration) × (volume of lymph produced).

The results of twenty-one experiments (all on dogs) are summarised in the

following table, and in order to facilitate comparison results have been calculated for unit time (1 hour) and unit body weight (10 kg.).

Table III. *Hourly output of thoracic duct lymphocytes (dog), in each case calculated for a body weight of* 10 *kg.*

No. of animal	Lymphocytes (in millions)	No. of animal	Lymphocytes (in millions)
4ES20	140·9	4ES34	240·4
4ES19	88·5	4ES35	209·6
4ES21	44·7	4ES36	406·1
4ES23	199·9	4ES37	194·4
4ES24	185·5	4ES38	147·7
4ES26	30·2	4ES39	875·4
4ES28	59·3	4ES40	121·5
4ES29	168·2	4ES41	149·8
4ES30	256·6	4ES42	138·7
4ES31	441·5	4ES65	221·5
4ES33	123·5		

Average hourly output (21 experiments)	=211·6 millions
Average output for 24 hours	=5078 millions
In proportion to body weight a human being weighing 70 kg. would have a total daily thoracic duct lymphocyte output of	35,546 millions

THE DAILY REPLACEMENT FACTOR

It will be observed, on examining Table III, that the thoracic duct lymphocyte output shows great variation in different animals. The question now arises: "Is this variability permanent, or not?" Does one animal normally and regularly have only 30 million lymphocytes per hour passing *via* the thoracic duct into the blood, while another has 875·5 millions, almost thirty times that amount? It seems unlikely, for a number of reasons, that this should be so. In the case, for example, of 4ES39 this would mean a lymphocyte production during the 24 hours of 21,000 millions, or 4·15 times the average figure. The significance of this may best be appreciated when it is compared with the total number of lymphocytes in the circulation.

The number of lymphocytes in the blood stream may be calculated if we know (*a*) the count per c.mm., (*b*) the blood volume. As to the *count per c.mm.*, Mayerson (1930), in sixty dogs, found the average count of "small and large mononuclears" in the blood to be 2680 per c.mm. If we assume that all these mononuclears are lymphocytes, which they almost certainly are not, then any error introduced will lead to an understatement of the position in calculations of the daily replacement of blood lymphocytes. In a series of my own (twenty-six experiments) the average count of blood lymphocytes was 3910 per c.mm. The *blood volume* may be calculated from the body weight. As is well known, there are marked differences between the results obtained by Welcker's, the carbon monoxide, and dye methods of estimation. Smith, Arnold and Whipple (1921), after a careful comparison of the various methods upon the same group of dogs, and an analysis of the possible sources of error, conclude that the

blood volume is 9·2 c.c. per 100 gm. body weight, so that a 10-kg. dog would have a blood volume of 920 c.c.

On the basis of these data (using Mayerson's figures for the blood lymphocytes) the blood of a 10-kg. dog contains 2465 million lymphocytes. Since the blood lymphocytes remain fairly constant over a period, the same number of lymphocytes must daily be entering the blood as are leaving it (Yoffey, 1933). Lymphocyte production on a scale as high as that found in 4ES39 would be sufficient to replace the blood lymphocytes in the average dog 8·5 times daily. Actually the blood lymphocytes in 4ES39 were above the average, being 6170 per c.mm., but even at this higher level they are being replaced 3·71 times per day.

The ratio

$$\frac{\text{number of lymphocytes daily entering blood } \textit{via} \text{ thoracic duct}}{\text{blood lymphocytes at beginning of experiment}}$$

will be referred to as the daily replacement factor, or D.R.F. One selects the blood lymphocytes at the beginning of the experiment as more truly representing the average level of the blood lymphocytes over a period, as during the course of the experiment the blood lymphocytes rapidly diminish, owing to the fact that such large numbers of thoracic duct lymphocytes, which in the intact animal would have been entering the blood stream and so have maintained the level of the blood lymphocytes, are now prevented from doing so. Consequently at the end of the experiment the blood lymphocytes are much lower than they were at the commencement, when the count does not differ from what it is in the intact animal, as the only factor which might have altered it is the anaesthetic, and the effect of this has been shown by previous workers to be negligible (Goodall and Paton, 1905; Latta and Ehlers, 1931).

4ES39 is the animal with the highest lymphocyte output of the series. The average figure for the series is probably of more universal applicability, and gives a truer picture of thoracic duct lymphocyte production over a period. The average daily output of lymphocytes from the thoracic duct of a 10-kg. dog is 5078 millions, and this means that the blood lymphocytes are normally replaced 2·06 times daily (using Mayerson's figures for blood lymphocytes). The lymphocytes therefore remain in the blood for 11·65 hours.

One further point may be noted in passing. In 4ES39 the thoracic duct lymphocyte production is 4·15 times the average figure, whereas the blood lymphocytes are only 2·3 times the average. In other words the level of the blood lymphocytes gives little indication of the degree of lymphocyte production. Lymphocytes may be entering the blood in vastly increased numbers, but if they are also leaving the blood rapidly, the level of the blood lymphocytes will not show any proportionate change. This point will be dealt with in detail in a further paper.

ARE THE THORACIC DUCT LYMPHOCYTES NEWLY FORMED CELLS?

The data which have been presented in the preceding paragraphs have all been based upon the assumption that the thoracic duct lymphocytes are cells which have been newly formed in the lymphoid tissues, and are entering the lymph for the first time. There is the alternative possibility to be considered, however, that they are not newly formed cells, but that there is a continuous circulation of lymphocytes from blood to lymph and from lymph to blood back again. The fact that we are not aware of any function which such a lymphocyte circulation might subserve does not, of course, constitute proof that it does not occur. The question as to whether the thoracic duct lymphocytes are newly formed cells or not is a fundamental one in all quantitative work based on thoracic duct lymphocyte estimations, and merits detailed consideration.

Thoracic duct lymph is the efferent lymph from the lower limbs, the abdomen, the left half of the thorax, left fore-limb, and left side of the head and neck. Under the conditions of the present experiments, the left cervical lymph trunk is divided, and the cannula is inserted well down into the duct, below the point of entry of the main efferent lymph trunk from the left fore-limb. Lymph, before it reaches the thoracic duct, passes through varying numbers of lymph nodes—in the present experiments chiefly those of the lower limbs and the large lymphoid mass at the root of the mesentery.

Now it has repeatedly been shown that whereas lymph which has not yet passed through any lymph nodes contains few or no lymphocytes, lymph which has passed through a lymph node is usually rich in such cells. Heyfelder (1851; quoted from Poirier, Cuneo and Delamere, 1903) seems to have been the first who "stated that the white corpuscles were much more numerous in the efferent than the afferent lymphatics". Brucke (1853) observed in cats and dogs which had been fed on a fat-free diet and killed while digestion was in progress, that the lymph in the lacteals (mesenteric lymphatics afferent to the large lymphoid mass at the root of the mesentery, and known as the Pancreas of Aselli or the glandula mesenterica magna) was quite clear, while the efferent lymph was turbid and full of corpuscles. Goodall and Paton (1905) found in the lacteals of the dog (i.e. afferents to the glandula mesenterica magna) an average count of 2160 lymphocytes per c.mm. (maximum 2600, minimum 1600), whereas in the efferent lymph they found an average count of 10,600. In the cat they found the count in the afferent lymphatics to be 1680, whereas it was 52,570 in the efferent lymph (collected from the receptaculum, into which the large efferent lymphatic of the glandula mesenterica magna opens directly). Davis and Carlson (1909) found that the lymphocyte content of efferent lymph was greatly increased by massage of the lymphoid tissues. In the cervical nodes they found the count was raised from 16,530 per c.mm. before massage to 51,700 after (average of four experiments), and

in the case of the thoracic duct lymph the lymphocyte count rose from 26,500 before massage of the abdomen to 73,330 after (average of two experiments). Florey (1927), in observations on the contractility of lacteals, noted only "the presence of an occasional white cell" in their lymph. He describes, on the other hand, the presence of large numbers of lymphocytes in the efferent lymph of the glandula mesenterica magna, and showed that splanchnic nerve stimulation still further increased their numbers, the average count in four experiments being 16,340 before stimulation and 37,950 after stimulation. Menkin and Freund (1929) found an average count of 18,000 in the efferent lymphatic vessel of one of the axillary lymph nodes of the normal rabbit. Haynes and Field (1931) found that, in the dog, lymph obtained from a vessel in front of the ankle contained an average of only 33 cells per c.mm., and they conclude: "The fact that lymph from the ankle, which has passed through no lymph nodes, contains practically no cells would indicate that normally the cells enter the lymph from the lymph nodes, and not by diapedesis from the blood nor from the tissue spaces." It is of interest to note that Drinker and Field (1933) come to a conclusion diametrically opposed to that which the figures of Haynes and Field (1931) warrant, for they say: "In our opinion, they" (i.e. the lymphocytes) "escape from the blood capillaries all over the body and re-enter the circulation *via* the lymph stream."

The available evidence, therefore, is strongly in favour of the lymphocytes in thoracic duct lymph being newly formed cells which have entered the lymph from the lymphoid tissues, on their way to the blood stream. Once the lymphocytes are in the blood stream, they do not re-enter the lymph in any appreciable numbers, for lymph contains few or no lymphocytes before it has passed through lymph nodes. The lymph in the mesenteric lymphatics may at times be an exception to this, but the exception is only apparent, for this lymph may already have passed through some lymphoid tissue in the wall of the intestine.

DISCUSSION

It must be borne in mind, in all quantitative work on thoracic duct lymphocyte output, that the figures obtained are minimal. Lymphocytes may enter the blood (*a*) from the thoracic duct, (*b*) from the right lymph duct, (*c*) possibly from the bone-marrow, (*d*) from the lymphocytopoietic tissues direct. As to (*d*), Schulze (1925) has described the existence of "stomata" in the post-capillary veins of lymphoid tissue, and believes that, through these stomata, cells from the lymphoid tissues have direct access to the blood stream. The number of lymphocytes which pass through these stomata into the blood is, of course, problematical; they cannot be directly measured. In the present experiments the only lymphocytes of which it has been found possible to obtain accurate quantitative estimations are those which enter the blood *via* the thoracic duct. If the number of lymphocytes which enter the blood from other sources are at all considerable, then the total

lymphocyte output of the body will be even greater than would appear from thoracic duct estimations alone. But even for the thoracic duct itself, evidence will be presented in another paper which seems to indicate that the figures obtained in these experiments are lower than they would be in the intact, actively moving, animal.

If it be true that the thoracic duct lymphocytes constitute only a part of the body's total lymphocyte production, it becomes all the more difficult to believe that the lymphocyte content of thoracic duct lymph could be maintained permanently at such a high level as is found in 4ES39. The alternative explanation would be that lymphocyte production does not take place at a constant and uniform rate, but is subject to marked fluctuation. If this view be correct, it would explain the great range of variation in thoracic duct lymphocyte production shown in Table III, and these data would then be interpreted as representing lymphocyte production in various phases. Over a longer period the average of Table III, namely 211·6 million lymphocytes per hour, would be a more representative figure.

Changes in lymphocyte output during the course of an experiment

That lymphocyte production is subject to fluctuation is further suggested (*a*) by the fact that evidence of change in lymphocyte production may be obtained during the course of an experiment, (*b*) by observations which have been made on the changing histological structure of lymph nodes.

The following experiment, for example, like the experiment in Table II, shows a progressive fall in the lymphocyte content of thoracic duct lymph (Table IV):

Table IV

4ES30. Dog. 13. iii. 35

Time	Lymphocytes per c.mm. lymph	Lymph in c.c. per hour
11.27 a.m.	15,000	Collection begun
12.27 p.m.	9,250	33·0
1.27 p.m.	7,100	35·0
2.27 p.m.	6,500	34·0

Table V, on the other hand, presents the results of an experiment in which the lymphocytes show a steady rise to a peak of 35,400, and then a sharp fall:

Table V

4ES39. Dog. 30. vi. 35

Time	Lymphocytes per c.mm. lymph	Lymph in c.c. per hour
1.40 p.m.	17,500	Collection begun
2.40 p.m.	24,000	15·0
3.40 p.m.	35,400	21·0
4.40 p.m.	21,500	23·0

The following experiment (Table VI) seems at first sight to show much the same phenomena as Table V.

Table VI

4ES75. Dog. 30. vi. 35

Time	Lymphocytes per c.mm. lymph	Lymph in c.c. per hour
12.25 p.m.	1300	Collection begun
1.25 p.m.	2500	30·0
2.25 p.m.	4600	24·0
3.25 p.m.	5000	15·0
4.25 p.m.	5200	22·0
5.25 p.m.	4300	52·0

If, however, one observes changes in lymph flow at the same time, it will be seen in Table VI that the rise in lymphocyte concentration from 1·25 to 3·25 is largely offset by the fall in the lymph flow. In the other experiments which have been quoted, on the other hand, the lymph flow has either remained constant while the changes in lymphocyte concentration were in progress, or else has increased together with the lymphocyte concentration.

The histological evidence

Finally, the conclusions of Maximow and Bloom (1930) on the histological evidence of cyclic activity in lymph nodes may here be quoted:

"The germinal centres are not constant structures. They are absent from the lymphoid tissues of the embryonic as well as of the senile organism. They appear and disappear periodically and pass through a series of cyclic changes. In the centre of an active, fully developed primary nodule there is a spherical clear area, the secondary nodule, which in man may attain a diameter of 1·0 mm. In the opposite condition, in the stage of complete rest, the germinal centre is quite inconspicuous. It consists of a small artery surrounded by a few pale nuclei of the undifferentiated syncytium in the centre of a follicle.... When a new period of activity begins—the duration of the stages is not known—the peri-arterial pale nuclei of the syncytium are mobilised and begin to divide mitotically."

SUMMARY

In twenty-one dogs a cannula was inserted into the thoracic duct, and both the flow of lymph and the concentration of lymphocytes measured.

After calculating the results for a standard body weight of 10 kg., the average hourly lymphocyte output was found to be 211·6 millions, with a maximum of 875·4 millions, and a minimum of 30·2 millions.

The entry of lymphocytes into the blood does not occur at a uniform rate, but shows marked fluctuation.

Thoracic duct lymphocytes are newly formed cells which have entered the lymph from the lymphoid tissues.

Thoracic duct lymphocytes enter the blood stream in numbers sufficient to replace the average blood lymphocytes of the dog 2·06 times daily.

The lymphocytes remain in the blood stream on the average 11·6 hours.

ACKNOWLEDGMENTS

My warmest thanks are due to Prof. C. M. West for his advice and encouragement, and to Prof. T. Graham Brown for his kind permission to perform in the Department of Physiology the experiments upon which this paper is based.

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THE HEAD PROBLEM IN CHORDATES[1]

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Utrecht

Roughly speaking two conceptions of the formation of the head region are possible.

(1) If we start from a bilateral symmetrical creature with ventral, dorsal and lateral sides, possessing a mouth at the anterior end and an anal aperture at the posterior end of the body, we can imagine that the principal sense organs with an associating nerve centre will gather at the anterior end in connection with the search for and examination of food. The head can be thus considered as the specially differentiated anterior end of the body.

(2) The head region of the adult form represents what has remained of the unsegmented larva and the trunk is formed by a kind of budding process. This conception needs qualifying since some unsegmented tissue forms a circular zone around the anus. In this, differentiation of the trunk segments takes place from behind forwards, the most caudal segment being thus the earliest. In either case the head region originally forms the whole body and the trunk arises as an appendix.

As we shall see the process of head formation in Chordates is much more complicated, agreeing in some respects with the first mode and in others with the second mode of development.

THE FORMATION OF GERM LAYERS AND ITS RELATION TO THE HEAD PROBLEM

The classical conception of Haeckel's Gastraea theory, with the extension given to it by the brothers Hertwig in their theory of the Coeloma, still plays an important part in the theoretical views of germ-layer formation. This influence is intelligible because the theory gives a comprehensive view of the whole question. The real cause of germ layer and coelom formation is the need for increase of surface, since in growth the mass increases by the cube and the surface by the square. Increase of surface may be secured by outgrowth. In animals outgrowth does occur in limb, tail, and gill formation, but in addition invagination occurs frequently, forming internal surfaces as

[1] In May 1935 I had the pleasure of delivering three lectures on the above named subject in King's College at the invitation of the London University. The following pages are a summary of these lectures. I wish to express here my hearty thanks to the editors of the *Journal of Anatomy* for giving me the opportunity to publish my theoretical considerations on this fascinating subject of general morphology.

in the formation of the enteric tube and its appendages, the neural tube, the majority of glands, the body cavity according to Hertwig's conception, etc.

I agree in general with this theory, but I think that Haeckel's and Hertwig's view that the starting-point of Metazoan development is the one-layered blastula becoming the two-layered gastrula is not correct. In my opinion the first differentiation occurs between the central and the superficial cells of the advanced morula stage.[1] The superficial ecto-entodermal cells possess an epithelial character, polarity of structure and form a continuous

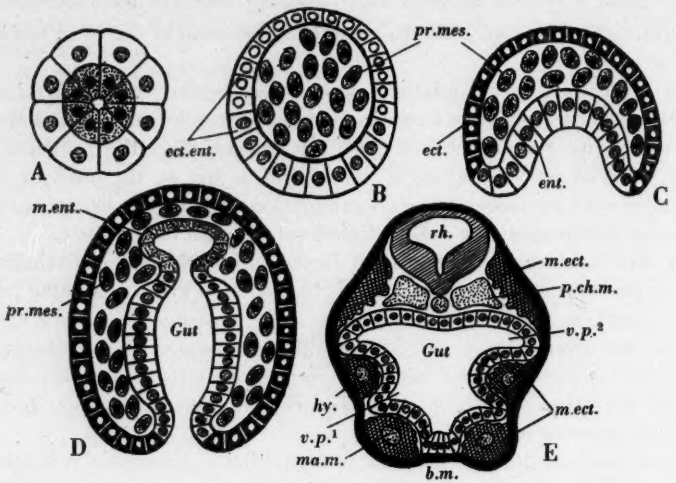

Fig. 1. Mesenchymula theory. A, two-layered morula with superficial and central cells; B, two-layered mesenchymula with beginning of differentiation of the superficial layer into ecto- and entoderm; C, three-layered mesenchymula with gut; D, older three-layered stage with mesentoderm formation (Echinoderms); E, mesectoderm formation in head region of Anamnian embryo.

sheet. The central cells on the contrary are isotropic, loosely connected with intercellular spaces between them.

Invagination of a part of the superficial cells does not form a true two-layered gastrula but a three-layered mesenchymula (see fig. 1 C). In the course of development the original primitive mesoderm may be completed by ingrowth or delamination of cells from the ectoderm or the entoderm (see fig. 1 D and E). Therefore in its final condition the so-called mesoderm contains elements of different origin, and I think Kleinenberg's famous dictum "es gibt kein mittleres Keimblatt" is quite correct. In the series of Metazoan animals only the ecto- and the entoderm may be compared; the composition

[1] There is some resemblance with Ray Lankester's "Planula hypothesis" and with Metschnikoff's "Parenchymula hypothesis", but these authors consider the central cells as the primordium of entoderm, while I think they represent the primordium of the primary mesoderm or mesenchyme in agreement with the conception of the Russian embryologist Schlater.

of the mesoderm is too variable for comparison. Anyone who studies the ontogeny of Invertebrates without preconceived ideas will come to the conclusion that the differentiation of the organism into three layers is fundamental in Metazoan development. The two-layered condition, i.e. the true gastrula, may be considered as an impoverishment, connected in many cases with the pelagic development of the egg.[1]

If we consider some further details of these processes in Invertebrates, we may state that in Molluscs and Echinoderms the mesenchymula as such changes into the larva. Beside the prostoma a new opening for ingestion or

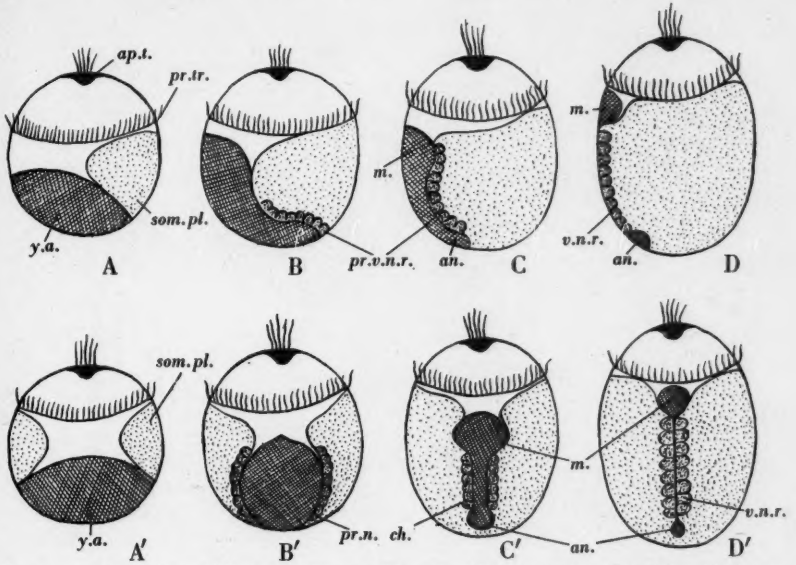

Fig. 2. Transformation of yolk area into mouth and anal plate in Annelids (after Davidoff). A–D, from the left side; A′–D′, same stages from the ventral side.

for egestion is formed. In Echinoderms the prostoma becomes the anal aperture, the mouth being a new structure; in Molluscs, on the other hand, the prostoma gives rise to the mouth and the anal aperture is a new formation. It is not my task to describe here the transformation of the bilateral larva into the adult asymmetrical Mollusc or into the secondary radiate Echinoderm. In both cases, however, we may homologise the bilateral larvae with the unsegmented stage of an Annelid.

In Annelids the formation of the larva, the well-known "trochophora" is quite comparable with the same process in Molluscs and Echinoderms, but in this case mouth and anus both originate in the prostoma. After the larval

[1] Where this condition is fixed in the adult, as in the case of *Hydra*, we have to do with a phenomenon of reduction connected with the transition from marine to fresh-water surroundings.

period a new centre of proliferation around the anal aperture comes into action and there is formed a number of segments in a caudo-cranial direction (see figs. 2 and 3). What remains of the trochophora gives rise to the original head segment and to the small post-anal telson of the adult, all the trunk segments being formed by the secondary centre of proliferation. In Annelids as in Arthropods the head region of the adult is composed of the original head segment and of a number of trunk segments.

In Chordates analogous phenomena seem to occur. As early as 1894 the Russian investigator Lwoff and the English embryologist Assheton raised objections to the classical conception of the Haeckel-Hertwig school which

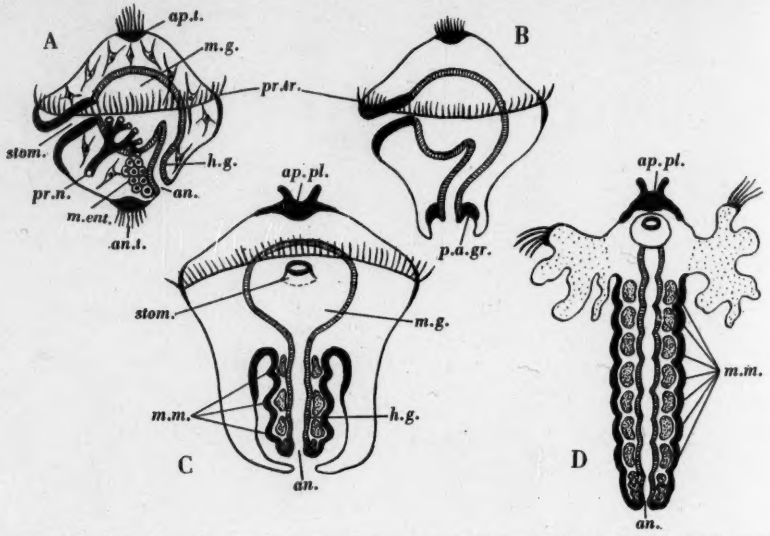

Fig. 3. Transformation of endotrochophora into segmented larva in Annelids (after Woltereck). A and B, from the left side; C and D, from the ventral side. In D the part of the trochophora cast off by the larva is dotted.

considered the mesodermal somites of the Vertebrate embryo as a series of entodermal pouches. This conception was largely founded on a false interpretation of the development of *Amphioxus* as represented by the investigations of Hatschek. It is a pity that in many leading German manuals of human embryology (e.g. Hertwig, Bonnet, Corning), the erroneous schematic figures of Hatschek are still used as the basis of Vertebrate ontogeny.

The theories of Gaskell and Delsman that the medullary tube has a stomodaeal character may be accepted in a general way. The mysterious connection of the medullary tube and the gut, viz. the so-called neurenteric canal, becomes in this way intelligible, and this connection may be interpreted as the primitive mouth of Chordates. If this supposition is correct, the closure of the blastopore in Annelids and Chordates shows a striking analogy. In both forms the

original blastoporic field is overgrown by ectoderm: in Annelids by an epibolic process; in Chordates the original epiboly is complicated by the process of chordulation. It assumes the form of a figure of eight, and the prostomal aperture is finally divided into the posterior anal opening and the anterior mouth (the neurenteric canal) (compare figs. 2, 5 and 6). In both cases the anterior opening loses its direct connection with its surroundings, and a stomodaeal tube with a secondary mouth is formed. In Chordates this tube acquires sensibility to external stimuli and becomes the most important part of the central nervous system (see fig. 4).[1] The longitudinal growth of

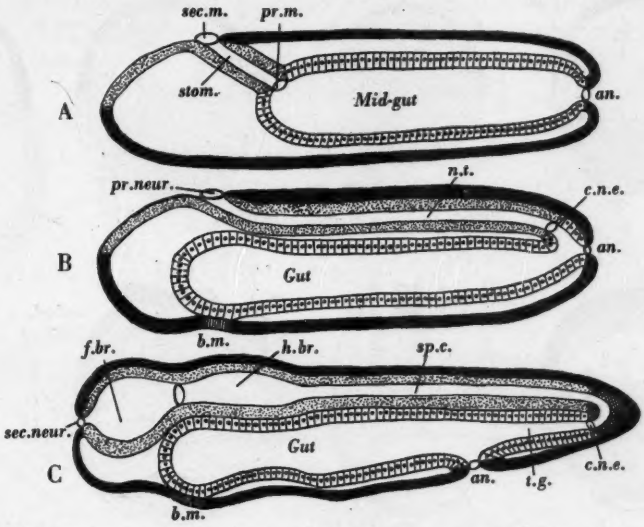

Fig. 4. Relation between entodermal gut and stomodaeum, e.g. neural tube. A, in Annelids; B, in *Amphioxus*; C, in Craniotes. The dotted part of the epidermis in A and B is incorporated into the fore-brain in C.

Annelids and Chordates, however, exhibits a striking difference. In the former the circular growth centre surrounds the anus. So this aperture always remains at the posterior end of the body, and a real tail, lying behind the anus, is never formed.[2] In Chordates, on the contrary, the growth centre lies

[1] If we compare fig. 4 B and C, and figs. 5, 6 and 7, we may observe that in Craniotes the animal pole is included in the foremost part of the brain. It is situated in the transverse brainfold (approximately at the posterior tubercle), while in Tunicates and in *Amphioxus* we find it at the ventral side of the head region entirely outside the neural tube. In Craniotes the micromeres between the animal pole and the primary neuropore form the archencephalon (according to v. Kupffer). In Urochordata and Cephalochordata this part of the brain is lacking. I shall not discuss the problem whether this represents a primary or a secondary condition. Therefore in reality the anterior tip of the chorda has the same position in *Amphioxus* and in true Vertebrates. In Tunicates it is receding in a posterior direction at a quite young stage (compare fig. 7 F and G).

[2] I exclude from the present discussion the telson, which remains rudimentary in Annelids but shows a fair development in Crustaceans.

for the most part in front of the neurenteric canal. Thus the dorsal part of the body can grow out behind the anal aperture and a real tail is formed (compare figs. 3, 4, 5, 6 and 7).

In Chordates the formation of the three-layered embryo is complicated by a secondary process, which we may call *chordulation*, as its predominant character consists of an invagination of material for the formation of notochord and somites. We can also use Assheton's term, *deuterogenesis*, laying

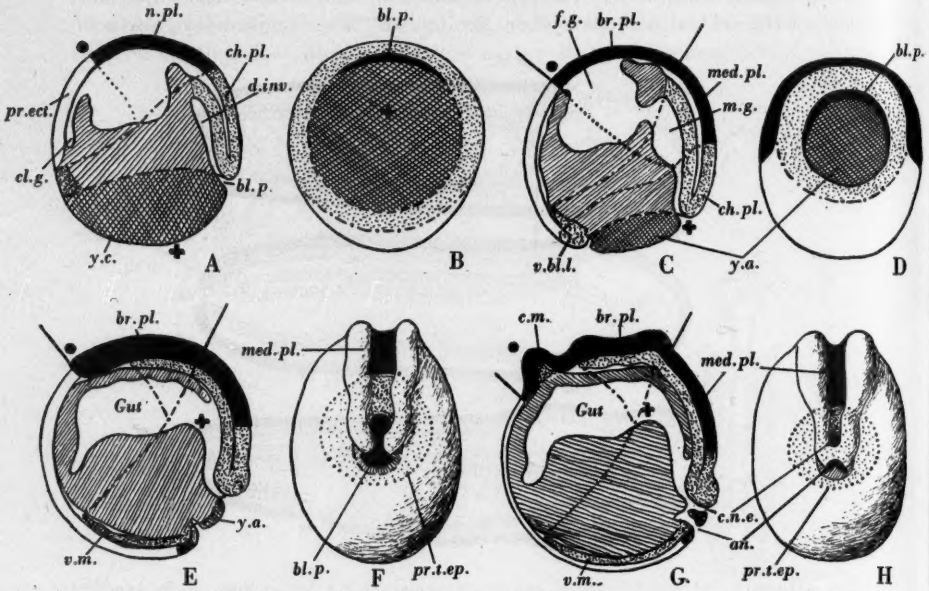

Fig. 5. Development of megalecithal amphibian egg. A, C, E, G, median sections. B and D, views from the vegetative pole; F and H, from the hind-side; ● animal pole; + vegetative pole.

stress on the secondary character of the process or Hubrecht's term, *notogenesis*, emphasising the fact that the axial organs of the back originate by this process. While at the surface of the Invertebrate blastula presumptive ectoderm (micromeres) and entoderm (macromeres) have a common boundary, these layers are separated in Chordates by a circular region of transitional blastomeres giving rise to the principal mesodermal organs of the embryo (compare figs. 2, 5, 6, 7 and 8).

On the other hand, there are several processes in Chordates which may be compared with the primary germ layer formation of Invertebrates, viz. the ectodermal epiboly at the ventral side, the "clivage gastruléen" of Brachet separating the ectoderm from the entoderm at the bottom of the segmentation cavity (compare fig. 5 A), the transformation of this cavity into the foremost part of the enteron in some cases (*Petromyzon, Rana, Megalobatrachus, Crypto-*

branchus, *Salamandra*, *Gymnophiones*, etc.), the dorsal closure of the entodermal gut and the delamination of the prechordal and ventral mesoderm from the entoderm (see fig. 5). Hubrecht has called these processes *cephalogenetic*, as they are comparable with the original head formation in Annelids. Assheton uses the term *protogenetic* because this phase of development in Chordates may be considered as the homologue of the primary germ layer formation in Invertebrates.[1]

In *Amphioxus*, the customary example of the Gastraea theory, we can clearly distinguish protogenetic and deuterogenetic processes, as has been pointed out by Cerfontaine in his exhaustive research on its early development (1906) (see figs. 6 and 7).[2]

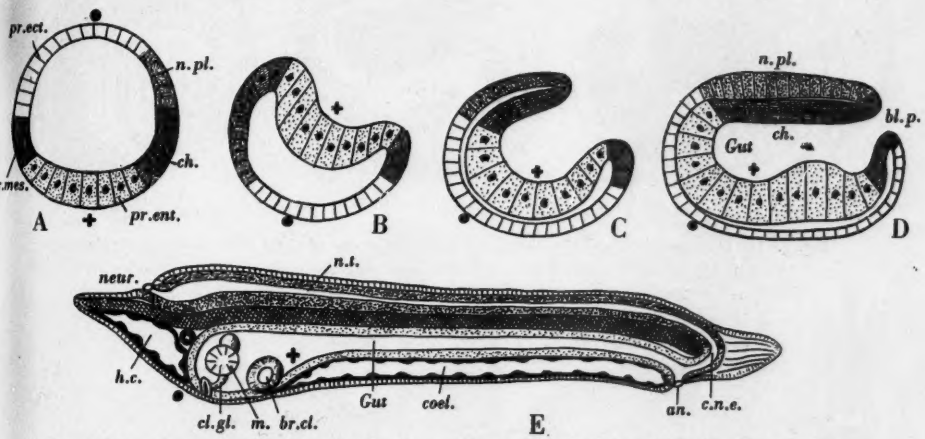

Fig. 6. Development of *Amphioxus* (after Cerfontaine and MacBride). A, blastula; B, beginning of gastrulation; C and D, further stages of three-layered gastrula; E, young larva with mouth and one branchial cleft.

In Tunicates these processes occur essentially in the same way, but the further development exhibits important differences (see fig. 7). While in *Amphioxus* the chordal plate penetrates as far as the tip of the snout, the anterior end of the chorda taking a pre-oral position, in Tunicates, on the contrary, chorda and parachordal mesoderm are expelled from the trunk region of the larva and give rise to the tail, which is only retained in *Appendicularia*. The adult sessile Tunicate may therefore be compared with the body of a Mollusc or of an Echinoderm or the unsegmented trochophora of an Annelid.

Passing to the true Vertebrates I shall limit myself to the germ-layer

[1] Apart from the question of precedence I think the use made by Brachet of the term *cephalogenesis* for the formation of the chordal part of the head and body must cause confusion.

[2] An indication that proto- and deuterogenetic processes may occur independently, is brought by Legros (1907) by his cases of asyntaxia blastoporalis.

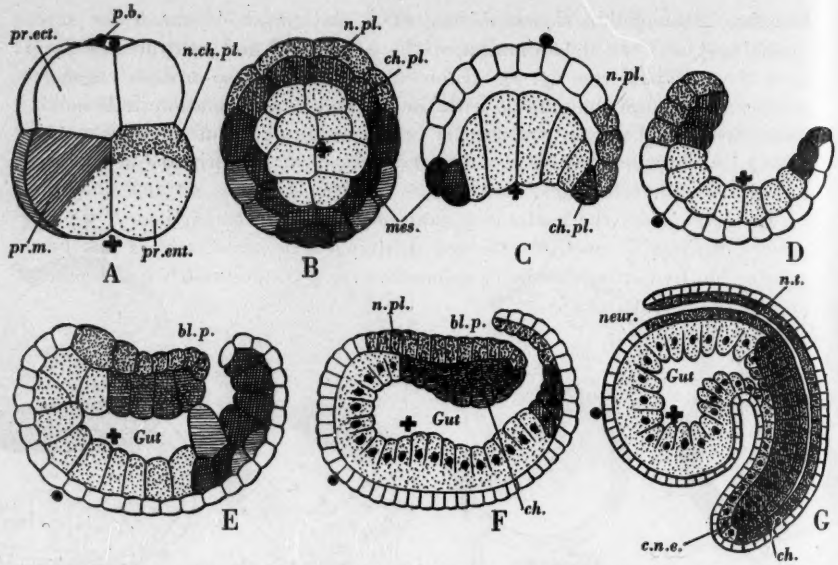

Fig. 7. Development in Tunicates (after Conklin). A, stage of eight cells viewed from the left side; B, beginning of gastrulation viewed from vegetative pole; C, median section of same stage; D–F, median sections of older stages; G, young tailed larva.

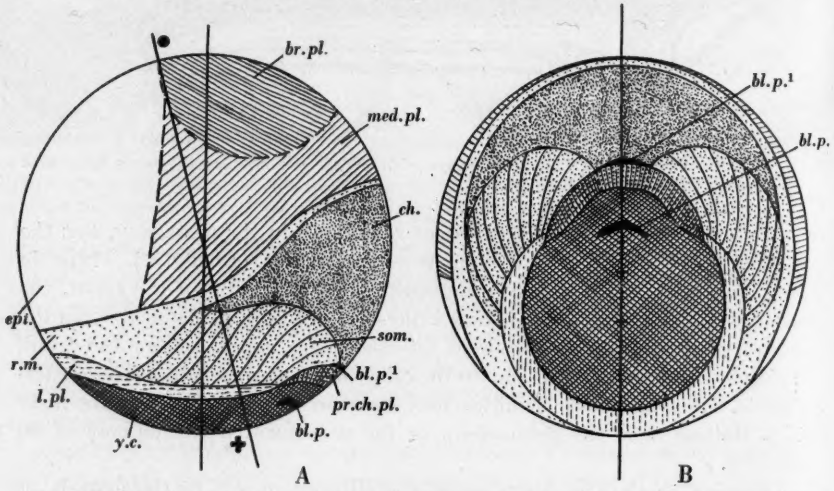

Fig. 8. Distribution of presumptive areas at the beginning of gastrulation in *Triton*, *Pleurodeles* and *Amblystoma* (after Vogt). A, from the left side; B, from the vegetative pole; *bl.p.* dorsal blastoporic lip in *Triton*; *bl.p.*¹ dorsal, blastoporic lip in *Pleurodeles* and *Amblystoma*; *r.m.* area indicated by Vogt as presumptive tail-bud. As a real tail-bud does not exist, it may be indicated as reserve material for the growth of mesoderm.

formation of holoblastic Anamnia, especially to that of Amphibians, which have become so well known through the excellent researches of German experimental embryologists—Spemann, Mangold, Vogt and others. As has already been pointed out the animal half of the blastula consists of micromeres and the vegetative half of macromeres. The two areas are separated by an annular zone of transitional blastomeres. The ventral half of the first zone

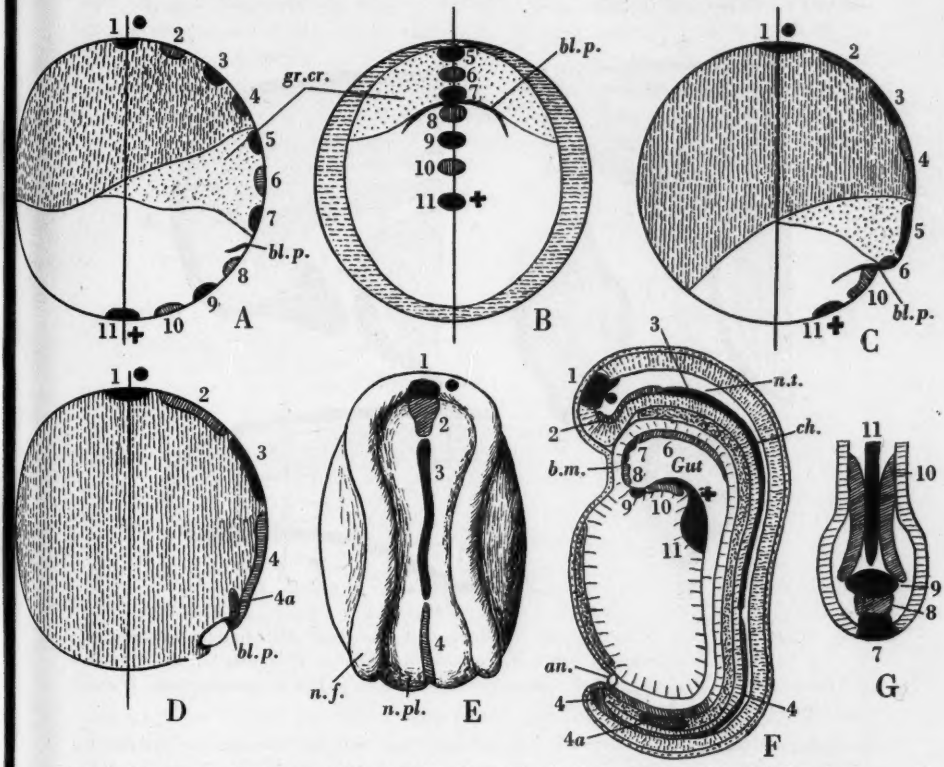

Fig. 9. Movement of colour patches in *Triton* (after Vogt). A, beginning of gastrulation from the left side; B, same stage from the vegetative pole; C and D, further stages from the left side; E, neurula from dorsal side; F, median section of young embryo; G, detail of the fore-gut of the same stage from the dorsal side.

may be considered as presumptive epidermis, the dorsal half as the presumptive medullary plate. The annular intermediate zone will give rise to the chordal plate and to the parachordal mesoderm wings. Its dorsal half covers the place of the grey crescent in the fertilised egg. This zone moves towards the vegetative pole being pushed on by the extension of the ectodermal area, and its connection with the entoderm is dissolved except with regard to its anterior and posterior portions (see fig. 9). Vogt has proved that the bulk of

the annular zone is used for the chorda and the axial mesoderm, the unsegmented lateral plate chiefly originating from the material of the ventral margin of the prostoma.

The formation of the definitive entodermal roof of the gut is formed by the macromeres growing upwards in the same way as in *Amphioxus*. In the fore-gut the whole cavity is immediately lined by entoderm, the dorsal plate never participating in this lining.

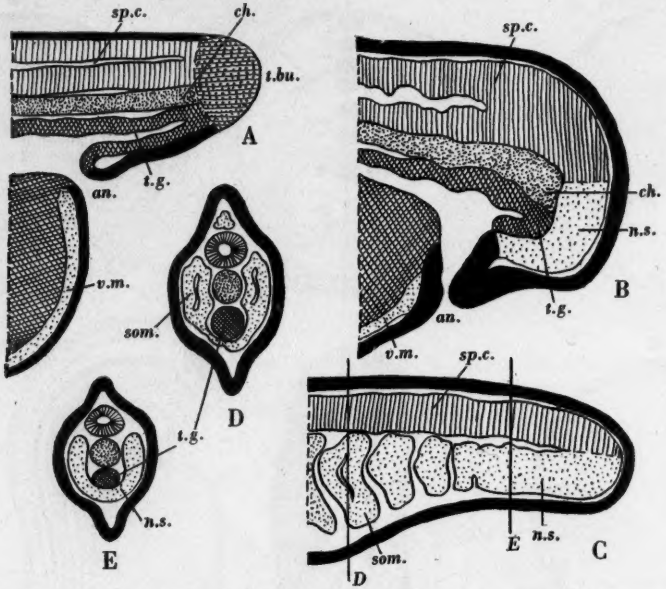

Fig. 10. Formation of the tail region in Amphibia. A, the classical scheme (median section); B–E, modern interpretation of tail formation in *Amblystoma* (after Miss Bijtel); B, median section at the beginning of tail formation; C, paramedian section of an older stage; D and E cross-sections of the same stage.

According to Vogt in all Amphibians the whole enteron is formed by invagination. I cannot agree with this opinion, though it may be correct for *Triton*, *Amblystoma* and *Bombinator*. In these species a stain at the original blastoporic groove is found afterwards at the liver invagination, and one at the vegetative pole will move to the middle of the enteric floor. In other cases,[1] however, especially when the eggs carry a large amount of yolk, the original ectodermal roof of the blastocoel is undergrown by yolk cells and this cavity is transformed into the fore-gut. The dorsal invagination unites with this cavity and only forms mid-gut and hind-gut. In this case the material which

[1] Viz. *Salamandra maculosa* (Grönroos), *Megalobatrachus maximus* (de Lange), *Rana* (Schultze, Brachet in some cases), *Pelobates fuscus* (v. Bambeke), *Alytes* (Gasser), *Gymnophiones* (Sarasin, Brauer).

is first invaginated at the dorsal lip may be expected at the anterior end of the chordal plate (compare figs. 5 and 9).

An analogous phenomenon occurs in the ectodermal covering. Here the presumptive brain plate and the epidermis of the branchial region are formed *in situ*. The formation of the medullary plate and the adjacent epidermis, on the contrary, is caused by a caudo-dorsal cell movement and by a considerable stretching in length of the whole "Anlage", connected with the growth of the dorsal blastoporic lip in caudal direction. In my opinion the formation of the brain plate and of the epidermis of the head region may be considered as a protogenetic process, as is the case with the invagination of the entoderm and the formation of prechordal and ventral mesoderm. The formation of the medullary plate, on the contrary, exhibits a thoroughly deuterogenetic character.

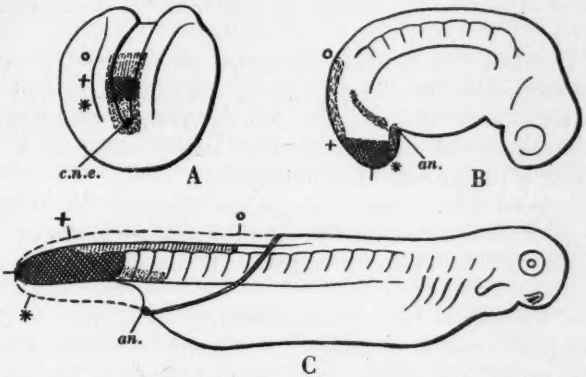

Fig. 11. Experimental research of tail formation in Amphibia (after Miss Bijtel). o, anterior limit of 1st colour patch; + limit between 1st and 2nd colour patch; * limit between 2nd and 3rd colour patch.

In the present state of our knowledge there is no reason to distinguish a special phase of tail formation after the closure of the prostoma; only a very great stretching of the dorsal embryonic region gives rise to the post-anal tail region and to the outgrowth of the pre-oral region of the head, both structures being of dorsal origin.[1]

[1] In former publications I have distinguished with Brachet a third embryogenetic process, viz. tritogenesis, urogenesis or cormogenesis, occurring after the closure of the prostoma and after its division into neurenteric canal and anal aperture. I adhered to the classical conception of the meristematic action of a terminal bud (teloblastema), an annular centre of growth around the neurenteric canal separating the material for the different organs of trunk and tail in caudo-cranial direction (see fig. 10 A). The remarkable researches of Miss Bijtel and Woerdeman dispose of such ideas. The principal facts are as follows: (1) a real meristematic tissue does not exist, (2) after the formation of the slit-like blastopore the entire material for the tail and for the caudal trunk region is lying in front of the blastopore between the medullary folds, (3) the tip of the tail can be fixed in this stage at a point lying $\pm\frac{1}{5}$ of the length of the neural plate in front of the

Summarising we can state the following points:

(1) The non-metameric parts of the Vertebrate embryo, viz. the gut with the splanchnopleura, the ventral body wall and the brain region with the anterior cephalic mesoderm, arise in the same manner as the three-layered, unsegmented larva of Invertebrates, the mesenchymula.

(2) The more or less metameric dorsal axial organs of the Chordate embryo arise by a developmental process characteristic of this group, viz. the chordulation, which possesses no homologue in Invertebrates. By this process the closure of the true enteric lips is delayed and a temporary roof of the gastral cavity, the chordal plate consisting of micromeres and transitory blastomeres, is formed. Afterwards the chordal plate is eliminated from the lining of the gut by the union of the entodermal rims.

(3) No special process of tail formation exists, the latter being only an expression of the excessive increase in length of the dorsal region of the embryo.

(4) In Tunicates (Urochordata) the deuterogenetic structures of the body are pushed away in caudal direction giving rise to the post-anal tail of the larva. The body of the adult animals, with exception of the *Appendicularia*, lose the tail region, and therefore the adult sessile animal can be compared with the body of unsegmented Invertebrates.

In Cephalochordata (*Amphioxus*), on the contrary, the deuterogenetic structures push on in an anterior direction and penetrate to the tip of the snout, originating a pre-oral chordal part of the body. A prechordal part is lacking.[1]

The true Vertebrates take an intermediate position, the anterior part of the chordal plate penetrating into the posterior head region, a second part forming the dorsal axial portion of the trunk region and the posterior part forming the tail. In this case only the pre-oral and prechordal part of the head region is entirely built up by protogenetic structures.

THE PROBLEM OF METAMERISM IN VERTEBRATES AND INVERTEBRATES

The conception of metamerism or segmentation has been based principally on morphological data in the group of Annelids. The original definition may be carried back to Haeckel, who considered a segment or metamere as a morphological individual of the fourth order, a repetition of the specific form. In his opinion the compound, segmented, adult animal must be compared with a cormus, a colony of individuals of the same asexual origin. Later

blastopore, this portion of the primitive medullary plate only giving rise to the tail somites, a few posterior trunk somites and the ventral epidermal covering of the tail (compare figs. 10 and 11).

[1] Strictly speaking the anterior end of the notochord is situated in *Amphioxus* at the same spot as it is in Craniotes, but the material for the archencephalon still forms part of the epidermis in Acrania (compare p. 519, footnote 1).

Hatschek may be described as the chief supporter of the theory of the cormus. Like the gastraea theory this conception is very suggestive, as it gives a simple explanation of the facts, but there are serious objections to it, which Arnold Lang has summarised in his famous study: *Beiträge zu einer Trophocoeltheorie.*

As we have already pointed out in the Introduction, the metamorphosis of the trochophora into the adult Annelid cannot be considered as a budding process like strobilation in Cestodes. In the latter case the centre of growth at the posterior end of the scolex separates the proglottides in an antero-posterior direction, the hindmost being the oldest proglottis. In Annelids, on the contrary, the circumanal growth centre is forming the segments in a postero-anterior direction. This mode of formation may be called intercalary, the hindmost segment being formed latest.

I imagine that in the Annelids the origin of the coelom and the segmentation of this cavity are connected with the wriggling mode of propulsion of the adult segmented worms. The coelom arises as a turgescent space functioning as a plastic support for the action of the cutaneous muscle tube. At the same time it gives firmness to the body and acquires connection with the ectodermal nephridial system. The segmentation of the coelomic space allows more complicated movements and increases their efficiency, as the contraction may be limited to a single element.

According to this conception the phylogenetic acquisition of the worm-like body form is not caused by the addition of a certain number of metameres to the original, non-metameric trochophora, but the elongation of the body, probably a consequence of the benthonic way of life, precedes metamerism, and the trochophora may be interpreted as an adaption to the planktonic, pelagic life of the larva. At all events the growth in length of the Annelid body is caused by the action of a circumanal growth centre forming in a caudo-cranial direction true metameres, viz. regular repetitions of whole portions of the body along the longitudinal axis.

In Annelids the principal organs of the body are represented in every segment, though functional and morphological differences occur between segments of different regions in the adult stage. The segmentation of the coelom and of the muscle tube may be considered as the primary phenomenon, secondarily causing the serial repetition of the other organs.

In the Vertebrates segmentation is quite different. Segmentation is now limited to the dorsal axial organs, especially to those of mesodermal origin. The primary unsegmented stage plays a far more considerable part in later development. It is not divided into a separate anterior and posterior region as is the case in Annelids, but forms a continuum out of which the whole ventral part of the body, viz. somatopleura, splanchnopleura, the entire gut, the archencephalon with the olfactory and optic sense organs, and the anterior head mesoderm, is formed. The secondarily formed metameric region of the body is originally limited to the axial, dorsal side of the body. Of course in

later stages of development the derivatives of the myomeres spread into the ventral, unsegmented part of the body.

Thus in Vertebrates real metamerism is present only in the muscle segment and in the nephrotome. The greater part of the coelom forms a continuous cavity without any traces of division. Only dorsal evaginations of this space penetrating the myotomes and giving rise to segmental stalks exhibit a serial character. The connection of these spaces with the dorsal muscle segments sufficiently explains their pseudometameric character. The primitive nephridial system (pronephros and mesonephros with their typically serial character) takes origin from the segmental stalks.

By analogy with our hypothesis of the origin of metamerism in Annelids we may suppose that myomerism and scleromerism in Vertebrates are connected with the fish-like propulsion of the primitive marine Chordates. Not only the tail but also the greater part of the trunk are used as organs of propulsion. Segmentation of the axial muscles raises their efficiency and makes possible the undulating movement of the body. This mode of propulsion requires an extremely flexible supporting apparatus as the notochord or a division of a more solid organ into small portions with mutual articulations, viz. the vertebral column. The stiff and unsegmented head region forms a point of support for the undulating movements of the body and serves as a kind of prow to cleave the water.

Originally the trunk portion of the central nervous system, viz. the medullary tube, the neural crest and the underlying supporting axial organ, the notochord, do not exhibit a serial character. Neuromerism and scleromerism are secondary phenomena, which are more or less dependent on the original myomerism.[1] The large axial blood vessels, viz. aorta, cardinal veins, and the gut, never show any trace of serial structure. So we cannot speak of a repetition of whole portions of the body because important organs such as the coelom and the vascular system do not exhibit a serial character. Therefore we might describe this seriality of dorsal axial organs in Vertebrates as pseudometamerism or dorsal metamerism.

Arising out of the above statement we may ask: "What is the morphological value of a special segment, and is there any reason to suppose that a metotic somite, No. X, has the same morphological significance in the whole series of Vertebrates?" I think no serious investigator would give an affirmative answer to the question put in this form, but many would argue in any concrete case that the significance of a special segment did not change essentially. Perhaps this curious contradiction is caused by the fact that the majority of morphological investigators are human anatomists, and in Mammals the number of cervical and thoracic segments is almost fixed. In this group a particular somite in most cases really represents the same morphological entity. If we pass, however, to other groups of Vertebrates we en-

[1] Of course the scleromeres are alternating with the myomeres and every scleromere develops out of a posterior and an anterior half of two succeeding metameres.

counter great variation in the number and arrangement of segmental structures in different parts of the body. If we adhere to the homology of every separate segment, we are obliged to drop the homology of the fore-limb, of the hind-limb, and of nearly the whole somatic muscular system in the different groups of Vertebrates. We have only to consult the classical publications of Fürbringer and Braus on the spinal nervous system of Selachians, Holocephali and Dipnoi, to see that the composition of the plexus cervicalis and brachialis and the number of spino-occipital nerves and of free spinal nerves innervating the fore- and hind-limbs show striking variations in allied forms. Rami pterygiales of the hind-limbs in *Ceratodus*, *Chimaera*, *Scymnus*, *Spinax*, and *Prionodon* are derived from the same spinal nerves, which give origin to the plexus brachialis and the free nerves of the fore-limbs in rays and skates. Besides, everyone knows that the stretching in length of the cervical region in many Reptiles and Birds is not caused by the increase in length of the cervical vertebrae, as is the case in Mammals, but by a considerable increase in the number of vertebrae. These examples will be sufficient to make it clear that the comparison of individual metameres in non-mammalian Vertebrates is quite impossible. We may suppose that the portion of the embryo available for segmentation is simply divided into the number of metameres needed for the different regions of the body.[1]

In spite of these facts many older anatomists attribute a fundamental significance to the phenomenon of metamerism in Vertebrates, and the distinguished Dutch anatomist, Prof. Bolk, formed a school of segmental anatomists amongst whom Van Rijnberk was the ablest. At the end of his life Bolk dropped his original conception of metamerism, but, as far as I know, he never published his change of opinion. That is the reason why I insist here on this fact. In a publication of 1908 on the innervation of the trunk and the limbs in Man we find the following sentences: "The segments may be considered as morphological units, being originally of the same value. The functions of the body as a whole are the result of the co-operation of the functions of the separate segments. In lower forms this co-operation is still very feeble, the individuality of the segments in these forms being very marked....The differentiation of complex organs leads to a diminution of the original individuality of the segments and an increasing co-operation and

[1] Of course we may cling to a fixed number of primary trunk segments and explain the variations in number and other deviations from our hypothetical scheme by supposing intercalated formation of new segments, but then we have no reliable criterion for the determination of the possible primitive number. Versluys has stated an analogous phenomenon with the reduction of the number of visceral arches in Selachians. In this case the common opinion supposes the reduction to occur at the caudal end, but this is impossible, as the last arch exhibits a special structure and possesses a close connection with the pericardium. Both characteristics are not lost in species with a reduced number of arches. According to Versluys, and I quite adhere to this opinion, only the first two arches (mandible and hyoid) and the last one are comparable in different species. The varying number of the other intermediate arches is determined by functional and topographical factors, e.g. the length of the head region and of the branchial region.

mutual dependence of the segments, but the existence of simple, intrasegmental reflexes makes it evident that even in Man the individuality of the segments is not completely lost....Now it is peculiar that the peripheral nervous system does not play an active part in the development of the body. On the contrary it shows a passive and very conservative character. A segmental nerve does not exert a determining influence on its area of extension, but when this region undergoes changes of the topography the nerve answers by elongation or ramification. A segmental nerve never yields a part of its territory to a neighbouring nerve and never tries to penetrate into a foreign territory."

This quotation from Bolk at the period of his most complete belief in segmental anatomy shows some peculiarities of the manner of thinking of segmental anatomists in general. Under the authority of Gegenbaur and Fürbringer a certain number of articles of faith are held. For example:

(1) *The nervous system is the most conservative part of the body.* We can retrace the phylogenetic wandering of special organs by the peculiar course of their nerves, and, in general, the history of special phenomena is recorded far better in nervous structures than in any others. On the contrary, everyone who studies the ontogeny and phylogeny of the nervous system in Vertebrates without preconceived ideas agrees that this system varies in structure and topography just as much as other organ systems.

(2) *The connection of the nerves with their terminal areas is fixed and unchangeable and exists from the very beginning of embryological development and differentiation.* I readily acknowledge that the connection between effector and receptor organs and their nerves is fairly constant; it is, however, changeable. Old connections may be lost and new ones may be gained. Everyone knows that in experiments on regeneration and transplantation, nervous connections with quite alien, heteroplastic tissue are formed. I refer for this phenomenon to the well-known investigations of Boeke, Braus, Detwiler, Harrison, Stone, Weiss *et al.* Even in normal development a shifting and overlapping of the areas of extension of spinal nerves regularly occurs. In his important research on the segmentation of the tail region in cat embryos Schumacher (1909) proved that the original concordance between spinal nerves and mesomeres is lost in this region.[1]

According to Erik Müller (1913) an analogous phenomenon occurs in the trunk and tail region of Selachians. Originally neuromerism and myomerism show strict conformity. However, in the course of development ramifications of the dorsal and ventral branches of the same spinal nerves penetrate into

[1] In the course of development the number of mesodermal segments increases and that of the spinal nerves, forming the cauda equina, decreases. Finally, the number of mesomeres surpasses 3–6 times that of the nerves. Thus every spinal nerve of the caudal region must innervate a number of segments. The case is still more complicated by the formation of a common nervous collector giving off ramifications to every segment. In this way the whole segmental pattern of the nervous system is lost in the tail region.

several myotomes, and plexiform connections between adjacent spinal nerves are formed giving rise to a dorsal and ventral collector nerve. Every myotome receives several nerves, and thus the area of distribution of a spinal nerve in the adult cannot be determined exactly.

The foundation of the morphological conception of the so-called dermatomes entirely relies on the distribution of the dermal branches of the spinal nerves, the cutaneous layers of the original mesomeres soon losing their metameric character and fusing into a continuous mass of mesenchyme. I have tried to make clear that the peripheral area of distribution of a special spinal nerve cannot be limited exactly, and in the following pages I shall try to prove that the same holds true for the central parts. Therefore in my opinion the dermatomes mainly have a topographical significance. This does not contradict the fact that in some cases they behave as physiological units (Sherrington).

NEUROMERISM

We may now return to the question whether neuromerism does really exist, or in other words, whether we may consider that the nervous system has been derived from a series of independent ganglia comparable to the rope-ladder system of Articulates, and gradually integrated by increased co-operation and interdependence.

The opposite opinion starts from the idea of a continuous nervous system without any trace of segmentation. The serial arrangement of the peripheral parts, especially of the myotomes, would impress on the nervous system a pseudo-metameric character shown by the serial arrangement of the spinal nerves and ganglia. In this view the importance of the central nervous system lies, from the very beginning, in the co-ordination of the functions and activities of different parts of the body. This system is the chief means of maintaining the unity of the organism. Longitudinal and transverse connections must therefore exist. Everyone familiar with the microscopic anatomy of the central nervous system will agree that, apart from certain important commissural connections, the fibre tracts travel in a longitudinal direction. In the majority of cases a ventral root is composed of fibres originating from motor nerve cells lying at different levels of the cord. Berkelbach van der Sprenkel (1924) has shown that in embryos of the hedgehog the composition of nerves is not fixed, but varies from one individual to another.

Therefore the neuromeric value of the spinal nerves is extremely questionable. Not only is every spinal nerve of multiple origin, but even the number and character of its components vary in different individuals of the same species. Besides, the ontogenetic data point to an original continuity of these macroscopical serial structures. The neural crest from which come the spinal ganglia and the dorsal roots is a continuous structure. The motor fibres arising from the ventral horn originally form an uninterrupted series. When the rudiments of the vertebral arches have developed, these fibres join together to leave the vertebral column between two arches. Sometimes inter-

mediate free motor fibres may be still observed between two successive ventral roots in older foetuses and adults. We cannot detect any sign of metamerism within the spinal cord itself. The ventral motor column is a wholly continuous structure. Differences in size of the transverse section of the column are the consequence of increased function of the corresponding terminal areas, e.g. at the level of the plexus brachialis and the plexus lumbalis. In young stages the medullary tube sometimes shows notches and flexures of a serial character, but it is evident that this phenomenon is caused by the pressure of the neighbouring mesodermal structures, viz. the mesomeres or the sclerometes. They are not the consequence of active differential growth of the spinal cord.

Now analogous facts occur in the rhombencephalon where we may observe seven or eight so-called rhombomeres in Selachians and Amniotes (Locy, 1895). These structures cannot be caused by myomerism or sclerometerism, as these do not occur over most of the hind-brain region. Von Kupffer, Neal, Charles Hill and others stress the significance of these facts as arguments for the primitive segmentation of the brain. They divide the whole brain region into fourteen or fifteen encephalomeres. In my judgment the so-called encephalomeres of the prosencephalon and mesencephalon are no such thing, but I admit that I cannot give a satisfactory explanation of the neuromerism of the hind-brain. There is a slight connection with the outgrowth of branchial nerves: the second rhombomere being connected in the chick with the trigeminus (V), the fourth with the acustico-facialis (VII–VIII), the sixth and seventh with the glosso-pharyngeus (IX) and vagus (X). This, however, leaves unexplained the rhombomeres 1, 3 and 5, which are not connected with branchial nerves.

Microscopic investigation, however, shows that correspondence between the implantation of the root and the nuclear origin of the nerves is lacking (see fig. 12). In Selachians (Neal) the trigeminus takes origin at the roof of the mid-brain and from the 2nd and 3rd rhombomeres; the nuclear origin of the facialis occurs from 4th to 7th rhombomeres; the glosso-pharyngeus from the 6th and 7th rhombomeres; and the vagus from the 7th and 8th rhombomeres. The origin of the branchial nerves is polymetameric, and the central connections of these nerves overlap. There is also a lack of correspondence between the external macroscopic seriality of the nerves and their central origin. The facts point for the most part to the conclusion that the serial phenomena in the nervous system are not fundamental but rather of a secondary nature. Neuromerism in the strict sense of the word probably does not exist.

BRANCHIOMERISM AND TRUNK-METAMERISM

With Gegenbaur (1871, 1888) and his school a generation of segmental theorists arose, who were largely imbued with Haeckel's conception of metamerism. These investigators, amongst whom Fürbringer is the most renowned, looked for rudiments of metamerism in the soft parts, especially in muscles

and nerves. Now there was a striking example of serial arrangement in the head region depending on the presence of serial gill clefts and gill arches. One can understand how these early investigators of the branchial region were inclined to suppose that branchiomerism and metamerism were identical. This supposition appeared to be supported by the embryological researches of Balfour, Wijhe, Hoffmann, Koltzoff and others, who detected a number of mesomeres in the parachordal mesoderm wings underlying the rhombence-

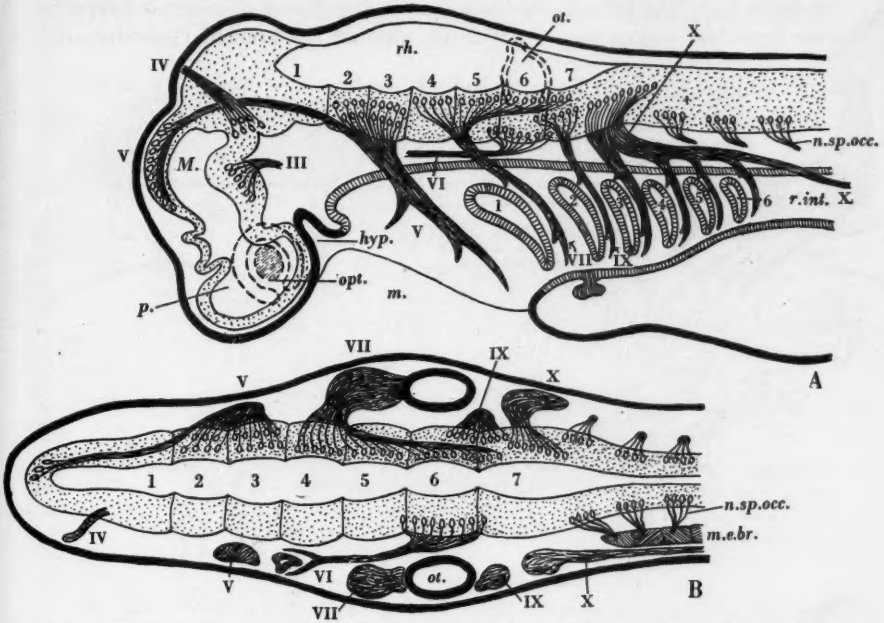

Fig. 12. The relation between rhombomerism and the origin of branchial nerves in Selachians (after Neal). A, from the left side; B, combined horizontal section of rhombencephalon, at the level of the origin of branchial nerves (right side) and of the ventral motor column (left side).

phalon. They believed that even the prechordal part of the head showed segmental mesodermal structures, viz. the so-called head cavities in Selachians and Sauropsida.

The presence of the notochord in a large part of the head region furnishes another argument for supporting an original conformity of structure of the chordal head region and the segmented trunk. The cranial nerves were regarded as the continuation of the series of spinal nerves. Of course a considerable difference in structure between these two kinds of nerves existed, but the extreme differentiation of the cephalic region might give a satisfactory explanation of this phenomenon. According to this opinion the vagus represented a number of spinal nerves, glosso-pharyngeus and facialis possessed the

value of one spinal nerve each, and the trigeminus probably was of compound structure too. As morphological research went on, difficulties soon arose. The principal muscles of the branchial region innervated by cranial nerves, the system of the constrictors and adductors, arise from the lateral plate mesoderm as do the smooth visceral muscles. They differ in this regard from the other voluntary striated muscles which take their origin from myotomes.

Functionally and histologically these muscles belong to the voluntary striated type. The lateral plate mesoderm being devoid of coelomic spaces in the branchial region, we do not know whether the branchial muscles arise

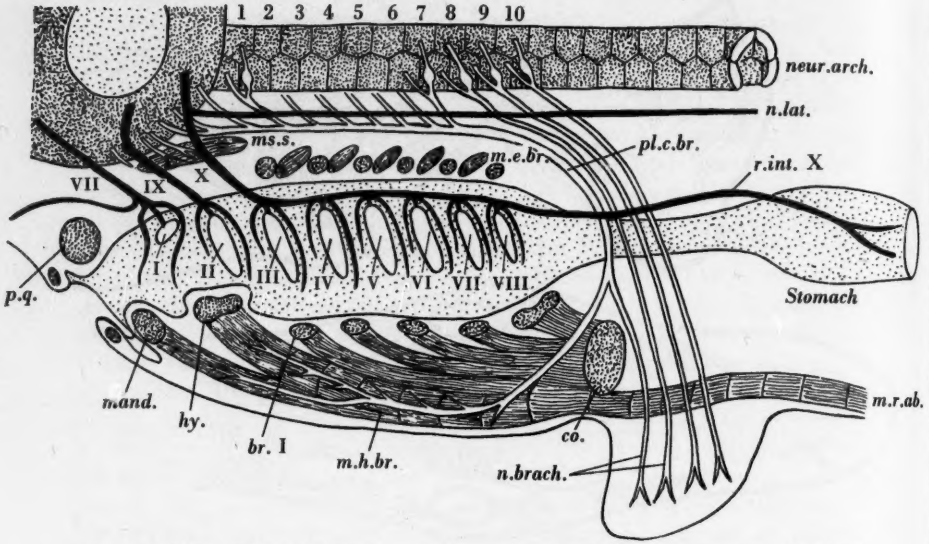

Fig. 13. Scheme of branchial nerves and plexus cervico-brachialis in Heptanchus (combined after the pictures of Fürbringer). *Narrowly dotted*, cartilage. *Widely dotted*, wall of the gut. *Hatched*, epibranchial and hypobranchial musculature. *Black*, cerebral, branchial nerves. *White*, spino-occipital and spinal nerves.

from the splanchnopleure or from the somatopleure. Now we may observe the curious phenomenon that a portion of the musculature of the branchial region, the so-called hypobranchial musculature connecting the shoulder girdle with the ventral portion of the visceral arches and a few epibranchial muscles (interarcuales, subspinalis) in Selachians, exhibit a thoroughly somatic character (see fig. 13). These muscles originate from myotomes or in any case from the dorsal parachordal mesoderm, and are innervated by true spinal nerves, i.e. by spino-occipital nerves. The arrangement of these spinal (spino-occipital) nerves differs from that of a cranial nerve so much that any idea of serial continuity between them is out of the question. Though ramifications of both systems cross at several spots, their elements remain entirely separated.

Branches of the cranial nerves innervating the ventral half of the branchial basket take the shortest way between the gill clefts; spinal elments, on the other hand, with an analogous terminal area take their way in posterior direction along the dorsal side of the branchial basket and unite to form the cervical plexus. They double round the caudal end of the basket and continue their way in anterior direction towards their areas of innervation. One gets the impression that:

(1) a complete separation between trunk material and branchial material is maintained, and

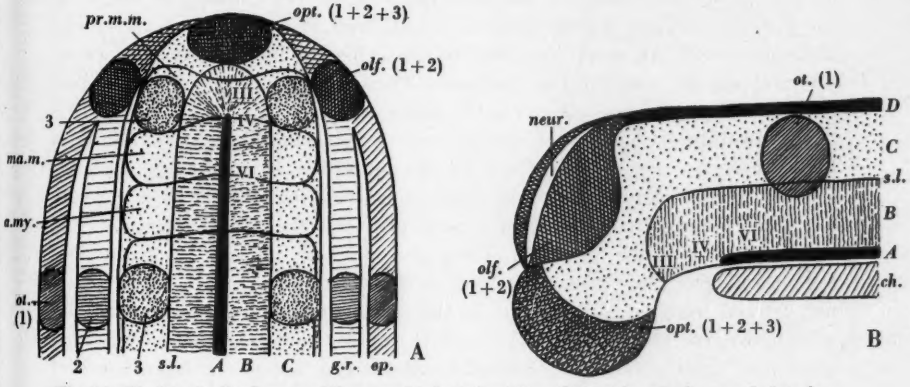

Fig. 14. The longitudinal areas of the neural tube (after His and Kingsbury). A, neural plate from the dorsal side with underlying parachordal mesoderm; B, neural tube from the left side (neuropore still present). *A*, floor plate (Bodenplatte); *B*, basal plate (Basalplatte); *C*, alar plate (Flügelplatte); *D*, roof plate (Deckplatte); 1, epidermal receptor; 2, afferent, sensory ganglion; 3, nucleus terminalis in neural tube.

(2) the branchial region has grown in caudal direction and has divided the trunk material into a smaller dorsal and into a larger ventral part, communicating at the caudal end of the branchial basket.

Of course this description may be reversed: the trunk material may be considered as growing in an anterior direction, meeting the caudal end of the branchial basket, and compelled by this obstacle to divide into a ventral and a dorsal part. The ansa hypoglossi in Mammals is a rudiment of this curious process.

Structure, development and topography of the cranial and spinal nerves exhibit striking differences. The division of the central nervous system adopted by His, and elaborated by Johnston and Kingsbury, nowadays is accepted by the majority of neurologists (see fig. 14). In the ventral mid-line of the neural tube the floor plate (Bodenplatte) contains only commissural fibres. The ventral portion of the lateral wall is formed by the basal plate, the dorsal one by the alar plate (Flügelplatte). In the dorsal mid-line the roof plate (Deckplatte) connects the alar plates of both sides. The basal plate contains

motor cells, the alar plate sensory nerve cells. The visceral nerve elements gather along the sulcus limitans which forms the boundary between basal and alar plate. In this way we may distinguish four longitudinal columns of ganglion cells, viz. in ventro-dorsal succession the somatic motor, the viscero-motor, and the viscero-sensory and somato-sensory (compare fig. 15 A and B). In the spinal cord the somatic motor column shows the largest cross-section; in the region of the rhombencephalon the visceral portions predominate.[1] Besides, in this region the longitudinal continuity of the somatic and visceral motor columns is disturbed. Sensory ganglion cells nowhere form a continuous structure. Therefore the hind-brain is the chief centre of origin of the visceral nervous system, and the spinal cord functions as centre of the somatic system. An exception must be made for the hypoglossus, the spino-occipital nerves, and for the abducens. These nerves may be considered as ventral spinal roots assimilated to the brain region.[2]

Other differences between cranial and spinal nerves are to be found in their ontogeny and topography. In Anamnia the typical cranial nerves (V, VII, IX and X) not only take origin from the dorsal neural crest but also from two longitudinal rows of epidermal placodes, the dorso-lateral and the epibranchial row (see fig. 16 A). These placodes only occur in the branchial region and give rise to the lateral-line system. The lateral lines of the trunk are derived from the primordium in the head region and are innervated by a cranial nerve (N. lateralis X). So it is obvious that a cranial nerve must remain in touch with the epidermal ectoderm and lies on the lateral side of the mesoderm. The dorsal root of a typical spinal nerve, on the contrary, originates from the dorsal neural crest only and takes its course on the medial side of the myotome (see fig. 16 B). The ventral root is formed by axons growing from nerve cells in the wall of the medulla and has no connection whatever with the dorsal neural crest.

As the continuity of the neural crest in head and trunk region is always present, there must occur a spot where dorsal mesoderm and neural crest cross (see fig. 16 C and D). Froriep has discovered this curious phenomenon, and we may agree with this author in considering this crossing point as the original boundary between head and trunk region, though in the majority of cases two or more trunk segments, forming the occipital region and being

[1] As is evident from fig. 15 A, the somatic sensory area is by far the largest in the hind-brain, but this area includes here the acustico-lateralis centre. Now the lateral line is undoubtedly of cephalic origin and penetrates secondarily in the trunk region. Presence or absence of this centre therefore furnishes a mark of distinction between cranial and spinal nerves.

[2] Perhaps this is also the case with the oculomotorius and trochlearis, but in my opinion there are serious objections to this idea. As both nerves possess their central nuclei in the mid-brain and are connected with prechordal, mesodermal structures, they must be considered as prechordal. They certainly contain viscero-motor fibres. Moreover, a dermal placode, giving rise to the ganglion ciliare, plays a part in the formation of III. These nerves, therefore, show a certain resemblance with the typical branchial nerves (V, VII, IX and X) of mixed visceral character. I think this matter is not yet settled.

connected with the hypoglossus, are assimilated into the head region in the course of ontogeny. The rudiments of more anterior head mesomeres lie at the medial side of the ganglionic mass. If there are still rudiments of spino-occipital nerves at this level, these nerves only possess ventral roots directly joining the corresponding myotomes. This also occurs in the abducens segment, the first parachordal somite, originating the M. rectus externus.[1]

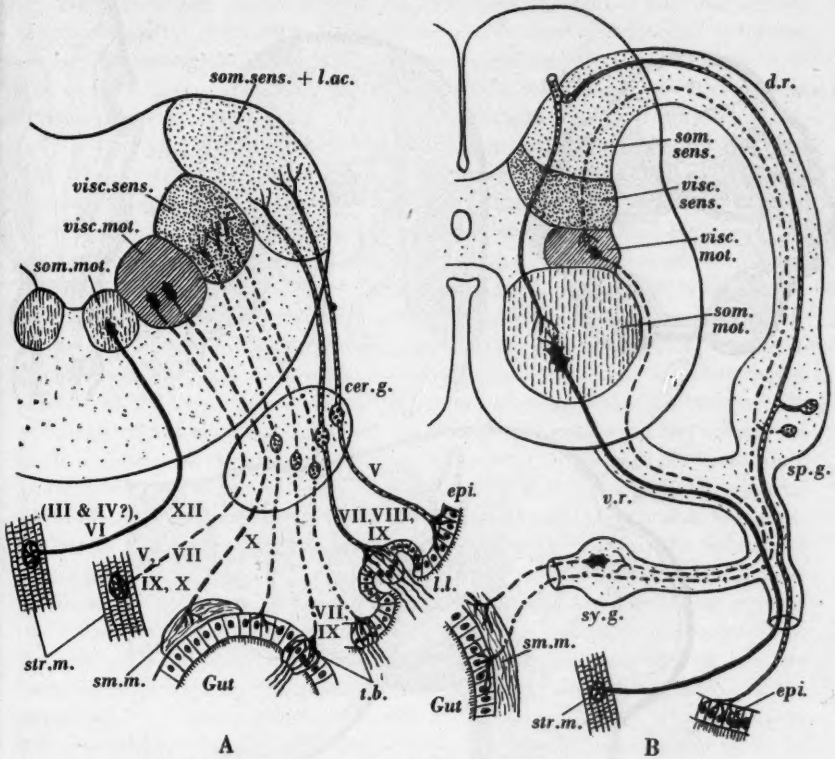

Fig. 15. Schemes of the structure of cerebral nerves (A) and of a spinal nerve (B) in Anamnia after Johnston.

[1] The majority of the authors hold the opinion that the abducens segment lies in front of the otic placode and may therefore be called pro-otic. Froriep, on the other hand, locates the otic placode in young stages at the anterior end of the chordal region. The considerable growth of the anterior prochordal head region, especially of fore- and mid-brain and of the anterior half of the rhombencephalon, would make this placode shift backwards while notochord and parachordal mesoderm remain *in situ*. As the abducens segment gives rise to the M. rectus externus of the eye, it will not only maintain its original position, but it will grow out in anterior direction to meet the eyeball. In Neal's schematic figures the nucleus of VI is represented in the 6th rhombomere, the otic capsule lying at the level of the limit between the 5th and 6th rhombomeres. This nucleus consequently shows a metotic position, while the externus somite is indicated behind the Vth cranial nerve at the level of the 3rd rhombomere.

According to the classical conception of head metamerism each cranial segment contains an encephalomere, a segmental nerve, a dorsal myotome, a lateral gill arch, the gill clefts showing an intersegmental position, and gill cleft derivatives (thymus buds, epithelial bodies). Numerical and topo-

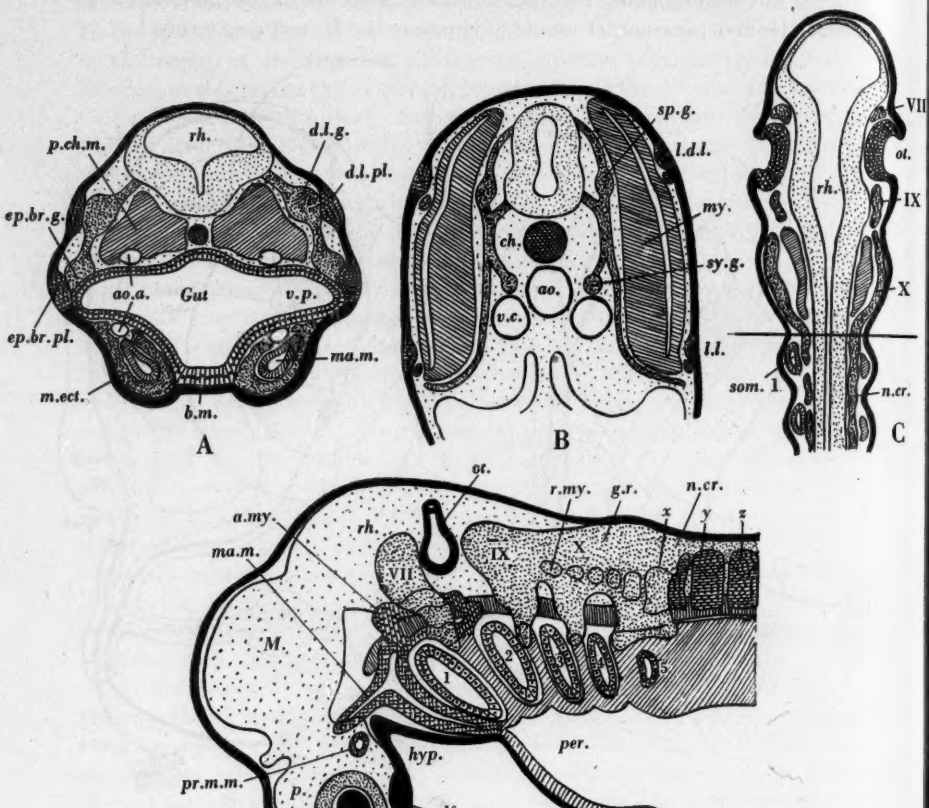

Fig. 16. Ontogeny of cerebral and spinal nerves in Anamnia. A, cross-section at the level of the first visceral pouch; B, cross-section through a trunk metamere; C, horizontal section through head-region of Acanthias embryo showing crossing of neural crest and parachordal mesoderm; D, scheme of head region of *Torpedo* (after Froriep) showing crossing of neural crest and mesoderm and relation between head mesoderm of different origin.

graphical correspondence between these elements is supposed to form the basis of the primitive plan of the head. There is an excellent publication on branchiomerism by Kingsbury (1926). It suffices to state that this correspondence is wanting, and support for it can only be found by admitting the

loss of one or more gill arches and gill clefts. There is no direct ontogenic evidence of any such loss. Koltzoff, a fervent adherent of the segmental theory, writes (1901): "No inner association is found between the developing visceral sacs and arches on the one hand and the mesodermal somites on the other, with the result that the segmentation of the branchial apparatus proceeds quite independently."[1]

Now let us give a short description of the development of the head region. In the majority of Anamnia the anterior part of head and fore-gut is formed *in situ* by protogenetic processes, though chronologically the development of the chordal part of the head occurs earlier. In some cases, however, e.g. in *Triton*, *Amblystoma*, and *Bombinator*, the material for the front wall of the fore-gut is lying in front of the dorsal blastoporic lip in the early gastrula, the whole enteric cavity being formed by invagination. In this case the wall of the fore-gut and the dorsal plate form a continuous layer from the beginning. In the other cases the anterior border of the dorsal plate and the wall of the fore-gut are widely separated at first, the secondary connection being caused by the ingrowth of the dorsal plate. In both cases, however, the wall of the fore-gut may be easily distinguished from the chordal plate by its abundance of yolk material. The anterior head mesoderm is delaminated from this entodermal wall. Originally it forms a continuous mass of mesenchyme uniting at its dorso-lateral ends with the parachordal mesoderm wings. Between the tip of the notochord and the thin medial portion of the prechordal plate a small area remains free of mesoderm. Here the floor of the brain plate touches the roof of the fore-gut. In the course of development the prechordal mass of mesoderm produces an unpaired anterior outgrowth and two pairs of lateral wings, the premandibular and mandibular masses. In Selachians and Sauropsida schizogenic cavities, viz. the premandibular and mandibular cavities, are formed in these mesodermal wings. The premandibular cavities of both sides are connected by a narrow canal, the mandibular remain separated.

The premandibular mesoderm gives origin to the majority of the eye muscles innervated by the oculomotorius (III), and the dorsal portion of the mandibular mesoderm gives rise to the M. obliquus superior, which is innervated by the trochlearis (IV). The ventral portion of this cell mass grows in ventro-caudal direction and forms the mandibular arch. An oblong area posterior to this arch remains free of mesoderm. Here the first visceral cleft will be formed. The hyomandibular cleft is a reliable boundary between the prechordal and chordal portion of the head region (see fig. 17). The prechordal portion contains the archencephalon (prosencephalon and mesencephalon), the olfactory and optic sense organs, the anterior head mesoderm and the portion of the fore-gut anterior to the hyoid arch.

[1] In connection with this question I should like to lay stress upon the fact that the serial arrangement of the visceral clefts as such does not involve true segmentation. (No one will call *Balanoglossus*, or a sessile Tunicate, segmented creatures, though in both cases the gill clefts are serially arranged.)

We have already mentioned the fate of the first parachordal mesomere, the abducens segment. The following indistinct anterior cranial mesomeres vanish in the course of ontogeny in a cephalo-caudal direction and become an unsegmented mass of mesenchyme. These transient structures are only found in Selachians; in other Anamnia and in Amniotes they are lacking. The lateral portion of the parachordal mesoderm grows in ventral direction and forms a continuous sheet round about the branchial region. In its ventral parts the paired primordia of the pericardial cavity will arise. Its antero-ventral border fuses with the postero-ventral one of the mandibular arch, surrounding in this way the hyomandibular cleft. So there occurs in the branchial region an intermediate stage showing no signs of segmentation, because the transient dorsal mesomeres have disappeared and visceral sacs are not yet present. In a somewhat older stage these entodermal sacs are formed and perforate the mesodermal sheet. Entoderm and ectoderm unite at these spots and finally the gill membranes rupture. The branchiomerism of the mesoderm therefore exhibits a quite secondary character and has no connection whatever with the dorsal mesomerism. The true metameric elements, which the branchial portion of the head region contains in the adult, form the epi- and hypobranchial musculature. Their corresponding spino-occipital nerves are connected with the third proper segmental portion of the head, the occipital region, and with the first trunk segments. They penetrate into the branchial region in later stages of ontogeny and remain completely separated from the true branchial elements.

Finally, we may draw attention to the relation between the vegetative and the cerebro-spinal nervous system and its bearing on the problem of the head formation. The simplest nervous system in Metazoa is found in Coelenterates. It consists of a ganglionic plexus between the ectodermal and entodermal epithelia. In the fibre strands of this network the nervous stimulus may progress in both directions. On the one hand it is connected with receptor sensory cells, on the other with effectors (muscle cells). The nervous function is ruled by the law of decrement, weak stimuli being limited to a small area, strong stimuli showing a larger extension. In the course of phylogeny the part played by the vegetative nervous net decreases and defined fibre tracts connecting receptors and effectors by a chain of neurones and synapses, where the direction of the stimulus is determined, come to prevail. In Invertebrates a dermal and visceral plexus persist. In Vertebrates only the latter is preserved.[1] Though the majority of vegetative nervous processes is limited to the visceral plexus, a certain connection with the superimposed cerebro-spinal system is maintained by the sympathetic and parasympathetic nerve tracts. The parasympathetic nerves arise from the cranial nerves, especially from the

[1] Woollard drew my attention to the structure of the cutaneous pain-perceiving system which quite resembles a primitive nervous net, and Boeke has observed similar peri- and intramuscular plexuses in striated muscle tissue (both researches have not yet been published in definitive form). These structures may be interpreted as remnants of the primitive nervous net.

ramus intestinalis vagi, and from the sacral nerves. They show an inhibitory character. According to the Dutch physiologist, Jordan, and others the primitive functions of the central nervous centre are inhibitory. The accelerating function of the orthosympathetic possesses a more secondary character. Phylogeny supports this view: in all Invertebrates and in *Amphioxus* and *Myxine* the orthosympathetic system is absent. If we regard the main part of the brain and the principal sense organs as derivatives of the non-segmented primordium of the body, we may then suppose the vegetative plexus and the parasympathetic to belong to this portion. Even the presence of the para-

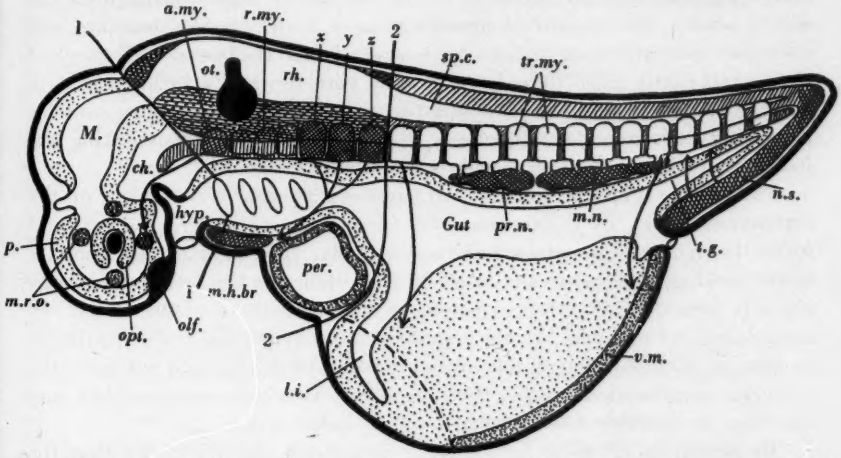

Fig. 17. Structural scheme of vertebrate body. *Black*, epidermis and its derivatives (lens, olfactory and otic placod$_e$, hypophysis), *Dotted*, protogenetic material belonging to the original unsegmented foundation of the body. *Hatched*, deuterogenetic material originating from the closure of the blastopore in the dorsal axial region of the body. *Hatched and dotted*, part of the neural tube of mixed origin (rhombencephalon). 1, limit between protogenetic and mixed region of the head; 2, limit between head and trunk region in the larva of Anamnia (in adult condition heart and pericard are removed to the pectoral region). The arrows indicate the spreading of the segmented muscles into the ventral region of the body. Of course the number of trunk and tail myotomes is quite arbitrary.

sympathetic tracts in the hind-part of the body is not so strange as the anal region and the procreative organs certainly belong to the vegetative part of the body.[1] Now in addition to this unsegmented fundament a segmented episoma develops at the dorsal axial side of the body. At first it chiefly possesses a locomotory function, it contains the segmented striated voluntary musculature with its co-ordinating nervous centre, the spinal cord. It also contains sensory elements with organs of touch in the skin and organs of

[1] The parasympathetic shows an analogous arrangement in Crustaceans (*Astacus*) where an anterior trunk arises from the cerebral end, para-oesophageal ganglia innervating the fore-gut, the stomach, the hepato-pancreas and the heart, while a posterior branch innervating the rest of the intestine is connected with the hindmost abdominal ganglion.

muscle sense. Simple reflex arches may be formed in this way. These spinal centres of directive movement may have been in relation with the existing central nervous apparatus of the unsegmented head region, i.e. with the brain from the very beginning. In the course of evolution the connection between the sensory apparatus of correlation in the head region and the co-ordinating motor apparatus of the episoma is strongly intensified.

In the course of phylogeny and ontogeny the episomatic structures spread over the surface of the whole body, covering the trunk with a coat of segmented muscles (the lateral trunk musculature), superseding in the terrestrial groups the original sense organs of the lateral line by organs of touch (in the widest sense). The segmented muscles impress their striated structure and voluntary character upon muscles of visceral origin in the buccal and branchial region and partly expel those in connection with the anal aperture and with the copulatory organ. This explains the mixed character of these muscles possessing such important functions as catching the prey, masticating the food, respiration, defecation, copulation and parturition.

Finally, the spinal nervous system impresses its serial arrangement on the orthosympathetic, its connection with the vegetative visceral plexus, and forms the chain of segmental sympathetic ganglia. In Selachians this structure is still lacking, in Teleosts and in all other Vertebrates the orthosympathetic chain is present. The vertebral ganglia of the sympathetic chain exhibit the same origin as the spinal, viz. the neural crest. At first spinal and sympathetic ganglia are almost in touch, but in the course of phylogeny and ontogeny the vertebral ganglia migrate away from the spinal. To a certain degree they may therefore be considered as derivatives of the spinal system.

Reflecting on all these facts I have formulated the hypothesis that the head and the body of the Vertebrate are not connected by both being formed out of the initial segmental structure of the whole body. They come into being and are connected through the primordial unsegmented fundament of the Metazoan body, upon which is superimposed in the case of Vertebrates a segmented dorsal episoma. This neoformation possesses chiefly a locomotory function, giving rise to the segmented striated musculature of the dorsal axial region, the organ of directive movement. Afterwards this axial musculature spreads over the entire external face of the trunk and penetrates into the primitive head region, viz. into the anterior oral region of the body where the most important sensory organs and the correlating and co-ordinating nervous centres are situated and where the branchial apparatus is formed. As this region functions as a firm prow for cleaving the water and as a *punctum fixum* for the undulatory movement of the segmented trunk, the episomatic structures in the head region soon lose their segmental structure, but they impress their histological and functional character upon the visceral muscles of this region. In the hypobranchial musculature, the episomatic and metameric character of the invading mesomeres and spinal nerves is retained, even in Mammals.

ABBREVIATIONS

a.my.	Abducens myotome (3rd myotome of v. Wyhe).
an.	Anus.
an.t.	Anal tuft.
ao.	Aorta.
ao.a.	Aortic arch.
ap.pl.	Apical plate.
ap.t.	Apical tuft.
bl.p.	Blastopore
b.m.	Buccal membrane.
br.	Branchial arch.
br.cl.	Branchial cleft.
br.pl.	Brain plate.
cer.g.	Cerebral ganglion.
ch.	Notochord.
ch.pl.	Chordal plate
cl.g.	Clivage gastruléen.
cl.gl.	Club-shaped gland.
c.m.	(Anterior) Cephalic mesoderm.
c.n.e.	Canalis neurentericus.
co.	Coracoid.
coel.	Coelom.
d.inv.	Dorsal invagination.
d.l.g.	Dorso-lateral ganglion.
d.l.pl.	Dorso-lateral placode.
d.r.	Dorsal root.
ect.	Ectoderm.
ect.ent.	Ecto-entoderm.
ent.	Entoderm.
ep.br.g.	Epibranchial ganglion.
ep.br.pl.	Epibranchial placode.
epi.	Epidermis.
f.br.	Fore-brain (archencephalon).
f.g.	Fore-gut.
g.r.	Ganglionic ridge (neural crest).
gr.cr.	Grey crescent.
h.br.	Hind-brain (deuterencephalon).
h.c.	Head cavity.
h.g.	Hind-gut.
hy.	Hyoid arch.
hyp.	Hypophysis.
l.ac.	Latero-acoustic centre.
l.d.l.	Linea dorsolateralis.
l.i.	Liver invagination.
l.l.	Lateral line.
l.pl.	Lateral placode.
M.	Mesencephalon.
m.	Mouth.
ma.m.	Mandibular mesoderm.
mand.	Mandibula (Meckel's cartilage).
m.ect.	Mesectoderm.
m.ent.	Mesentoderm.
m.e.br.	Epibranchial musculature.
med.pl.	Medullary plate.
mes.	Mesoderm.
m.g.	Mid-gut.
m.h.br.	Hypobranchial musculature.
m.m.	Metameres.
m.n.	Metanephros.
m.r.ab.	Musculus rectus abdominis.
m.r.o.	Ocular muscles.
ms.s.	Musculus subspinalis.
my.	Myotome.
n.brach.	Nervi brachiales.
n.ch.pl.	Neurochordal plate.
n.cr.	Neural crest.
neur.	Neuropore.
neur.arch.	Neural arch.
n.f.	Neural fold.
n.lat.	Nervus lateralis.
n.pl.	Neural plate.
n.s.	Neuralstrang (neurenteric cord).
n.sp.occ.	Spino-occipital nerves.
n.t.	Neural tube.
ot.	Auditory organ or vesicle.
olf.	Olfactory organ and nerve.
opt.	Optic nerve and vesicle.
p.	Prosencephalon.
p.a.gr.	Perianal centre of growth.
p.b.	Polar bodies.
p.ch.m.	Parachordal mesoderm.
p.ch.pl.	Prechordal plate.
per.	Pericard.
pl.c.br.	Plexus cervico-brachialis.
p.q.	Palato-quadratum
pr.ect.	Presumptive ectoderm.
pr.ent.	Presumptive entoderm.
pr.mes.	Primitive mesoderm.
pr.m.	Primitive mouth.
pr.mes.	Presumptive mesoderm.
pr.m.m.	Premandibular mesoderm.
pr.n.	Protonephridium (in fig. 17 pronephros).
pr.neur.	Primitive neuropore.
pr.t.ep.	Presumptive tail epidermis.
pr.tr.	Prototroch.
pr.v.n.s.	Presumptive ventral nerve strand.
rh.	Rhombencephalon.
r.int.	Ramus intestinalis.
r.m.	Reserve material.
r.my.	Rudimentary cephalic myotomes.
s.l.	Sulcus lateralis.

sec.m.	Secondary mouth.	*tr.my.*	Trunk myotomes.
sec.neur.	Secondary neuropore.	*v.bl.l.*	Ventral blastoporic lip.
sm.m.	Smooth musculature.	*v.c.*	Vena cardinalis.
som.	Somite.	*visc.mot.*	Viscero-motor centre.
som.mot.	Somato-motor centre.	*visc.sens.*	Viscero-sensible centre.
som.pl.	Somatic plate.	*v.m.*	Ventral mesoderm.
som.sens.	Somato-sensible centre.	*v.n.s.*	Ventral nerve strand.
sp.c.	Spinal cord.	*v.p.*	Visceral pouch.
sp.g.	Spinal ganglion.	*v.r.*	Ventral root.
stom.	Stomodaeum.	*x*	1st occipital myotome.
str.m.	Striated musculature.	*y*	2nd occipital myotome.
sy.g.	Sympathetic ganglion.	*y.a.*	Yolk area.
t.b.	Taste bud.	*y.c.*	Yolk cells.
t.bu.	Tail bud.	*z*	3rd occipital myotome.
t.g.	Tail gut.		

SOME IMPORTANT PAPERS ON THE HEAD PROBLEM AND ON METAMERISM

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1871. Carl Gegenbaur. "Über die Kopfnerven von Hexanchus und ihr Verhältnis zur Wirbeltheorie des Schädels." *Jena. Z. Naturw.* Bd. vi.

1874, 1878. B. Vetter. "Untersuchungen zur vergleichenden Anatomie der Kiefer- und Kiemenmuskulatur." *Jena. Z. Naturw.* Bd. viii and xii.

1876, 1878. F. M. Balfour. "On the development of the Elasmobranch Fishes." *J. Anat. and Physiol.* vols. x and xii.

1877. Ernst Haeckel. *Biologische Studien.* H. 2. *Studien zur Gastraea-theorie.* Jena.

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THE SURGICAL ANATOMY OF THE NECK IN RELATION TO SEPTIC LESIONS

AN INVESTIGATION OF THE CERVICAL CONNECTIVE TISSUE

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INTRODUCTION

It is, perhaps, still insufficiently recognised that the presence of pus within the neck calls for the exercise of the utmost skill and judgment based upon a sound knowledge of the surgical anatomy of the part. Pus may collect in the neck from a variety of primary foci, and spread of sepsis is apt to occur uninterpreted or even unnoticed. The "wooden" oedematous neck frequently presents a baffling picture no less difficult of diagnosis than that in which deep pus reveals little or no external evidence of its presence.

The purpose of this paper is to emphasise the importance of a subject which, it is felt, has received insufficient attention, and to place on record the results of certain anatomical investigations designed to elucidate the problem of the routes taken by and the limitations of infective processes. The scope of the enquiry is confined to a study of surgical anatomy and was prompted by Kanavel's(1) and Koch's(2) work on the hand. As a result of clinical experience, injection experiments and serial sections, these workers were able to place the surgery of the hand on a sound footing. As to the present work, nothing revolutionary and little that is new is claimed, for Mosher(3, 4), Beck(5), Ford(6), and others have already tackled the subject from the clinical point of view and have drawn attention to certain major principles which the writer has, in the main, been able to confirm experimentally, although with certain modifications.

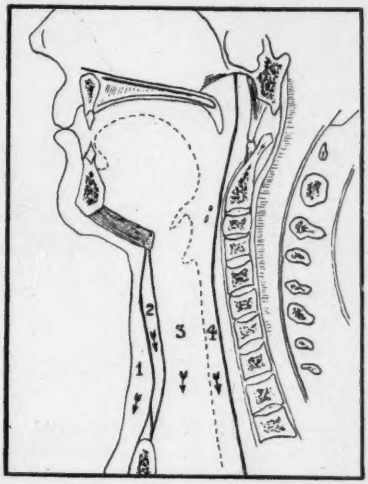
Mosher, in 1920 and later in 1929, drew attention to the importance of the cervical fascia in limiting and directing the spread of pus, and from Woolsey's *Applied Anatomy* briefly described three layers as follows:

(1) *The Superficial Layer* splits to enclose the sternomastoid and the trapezius muscles in a sheath. Above the hyoid bone it forms a sheath for the submaxillary gland and above this it is continuous with the parotid and masseteric fascia. It also makes the stylo-maxillary ligament.

(2) *The Middle Layer*, which is attached to the hyoid bone, covers the muscles above it which form the floor of the submaxillary triangle, and is attached to the mylo-hyoid ridge. Below the hyoid it forms a sheath for the depressors of the hyoid bone. It works its way downwards as far as the pericardium and the sheath of the axillary vessels. From the deep surface of the middle layer are given off expansions which make a sheath for the trachea, the thyroid and great vessels of the neck.

(3) *The Deep or Pre-vertebral Layer* covers the prevertebral muscles and is attached laterally to the cervical transverse processes, where it is continuous with the sheath of the scalenus anticus muscle and that of the brachial plexus. Inferiorly it is continuous with the sheath of the subclavian and the axillary vessels. It completes the carotid sheath posteriorly and marks the posterior boundary of the pharyngomaxillary fossa. It lies behind the oesophagus and the pharynx.

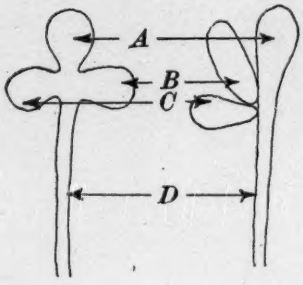
Mosher pointed out how, in relation to these layers, four fascial compartments in which pus might accumulate seemed to exist. The first lay superficial to the superficial layer, between it and the superficial fascia, and contained the platysma and the external jugular vein. From this space pus could reach the surface with relative ease. The second was between the superficial and



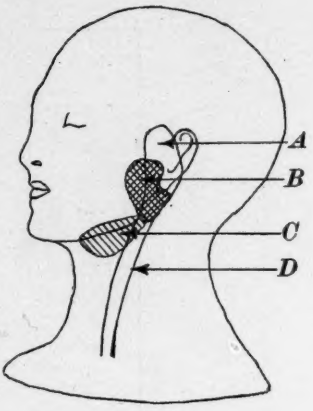
Text-fig. 1. Mosher's three fascial layers and four spaces.

middle layers and contained the submaxillary glands above and the infrahyoid muscles and anterior jugular veins below. The two parts did not communicate because of the attachment of the superficial layer to the hyoid bone, and pus was prevented from entering the thorax by the attachment of the middle layer to the sternum and clavicles. The third compartment was described as containing the visceral group of structures (trachea, oesophagus and thyroid), the great vessels and the lymph nodes, and was said to be covered by both the superficial and middle layers of fascia so that the presence of pus was obscured and the latter had great difficulty in working its way to the surface. Lastly, the fourth compartment lay behind the pharynx and oesophagus. Below the level of the hyoid it ran directly into the third compartment, whilst above that level it was represented as lying behind and at the side of the pharynx, in which latter position it was called the pharyngo-maxillary fossa, and this was said to be "continued into the neck as the carotid sheath".

These observations of Mosher's, based as they were on considerable clinical experience, represent the first real effort at unravelling the tangled problem of cervical cellulitis. Perhaps his most important contribution was the emphasis he placed on the importance of the pharyngo-maxillary (the anterior part of which is referred to later in this paper as the lateral pharyngeal) and submaxillary spaces, and the frequency with which jugular vein thrombosis occurred in many different types of neck suppuration in which adequate drainage had been delayed too long. Mosher considered that such thrombosis was due to direct contact of the vein with pus either below the level of the hyoid bone or in the pharyngo-maxillary space.



Text-fig. 2.



Text-fig. 3.

Text-fig. 4.

Beck's conception of a cervical trifolium. *A*, pharyngo-maxillary space. *B*, parotid space. *C*, submaxillary space. *D*, carotid sheath.

Beck, in reaffirming the importance of the above spaces, elaborated the matter further and, from the point of view of deep suppuration in the neck, visualised the upper cervical region as the leaves of a trifolium of which the carotid sheath (i.e. the connective tissue immediately surrounding the carotid artery and jugular vein) was the stalk. The three "leaves" were the submaxillary, the parotid and the pharyngo-maxillary spaces, and are represented in the accompanying diagrams copied from his paper (text-figs. 2, 3, 4).

He believed that the four regions were in more or less direct intercommunication, and this view has, in large part, been confirmed in the present research so far as the upper three spaces are concerned, although in regard to the "carotid sheath" certain reservations are made. In any event, there seems to be no doubt that, clinically, pus can pass from either the parotid or submaxillary regions into the lateral pharyngeal region and thus accumulate very deeply, external to the constrictor muscles and internal to the parotid gland and the internal pterygoid muscle, and that, as a result of such suppuration, thrombosis of the jugular vein is prone to occur. The latter, in fact, has

been known to necrose, so that it comes to lie in an abscess cavity. Some observations upon the probable mechanism of jugular infection are made below.

Ford agrees in general with the conception of a three-leaf arrangement, but believes that pus burrows down from the pharyngo-maxillary space around and not in the carotid sheath.

The attention given to the anatomy of cervical infections has, on the whole, been inconsiderable. The subject is of importance, for these infections are often serious and the mortality is high.

With the striking exception of Frazer's(7) characteristically careful work, there has been a lack of interest in the anatomical side of the subject. In some degree this may be due to the scepticism which has grown up about many of the fascial planes and laminae that have been described hitherto in so much detail. It has been contended that these fascial planes are artefacts due to formalin fixation, and a dissector who wishes can easily demonstrate other planes according to the plan of his dissection. Testut(8) illustrates no fewer than five different conceptions of the fascia in the styloid region, yet none is definitely accepted as fact. This lack of belief is further reinforced by reflecting on the embryological origin of connective tissue sheets and planes, etc. Everywhere the irregularity of surfaces, the inevitable corners and crevices, the uneven contours produced in the course of growth, are packed and filled with the tissue which becomes the fascia, etc., of ordinary anatomy. To these doubts must also be added the statements of surgeons that they do not encounter, in the living, fascial planes and laminae which have been erected and described by dissectors. Pus is not an inert fluid; it is capable of making its own way through tissues, but it may be that in some degree it is guided by planes, spaces and other anatomical structures.

Thus it is necessary that the existence and extent of spaces and boundaries should be demonstrated by adequate methods in unfixed subjects. Further, it must be shown that the spread of infection in the neck is strongly influenced if not determined by these spaces and their boundaries. If investigation shows that the spread of infection is so governed, then the hope of improving surgical treatment might prove well founded.

SOME GENERAL CONSIDERATIONS

The neck, looked at in a general sort of way, may be regarded as consisting of four subdivisions. These units are:

A. *Vertebral* (including the vertebrae with their muscles).

B. *Visceral* (including the thyroid, larynx, trachea and oesophagus below and the mouth and pharynx above).

C. *Great vessel* (carotids and jugular).

D. *Vaginal* (the trapezius and sternomastoid muscles and, as will be shown later, the strong connective tissue between them).

If it is a fact that true connective tissue condensations exist, it might be

expected that they would be found where movement is least or absent, where stability is required or between groups of muscles of similar function.

Where movements of one part upon another are required, or where two different kinds of movement go on side by side, it might be reasonable to expect no close cohesion by connective tissue condensations, but rather a looseness of the fascial arrangement to promote ease of independent motion. In such positions pus might be expected to accumulate and spread.

Fat is an inert substance with a poor blood supply and is found only where there is relative rest. It does not occur, like the loose areolar tissue of the preceding paragraph, in places where one structure moves freely over another. Nevertheless, its poor vitality and the fact that it frequently contains lymphatic nodes suggest that it may easily be invaded by pus; and this we know to be a common clinical occurrence. In the neck, fat deposits are found in the following situations:

Subcutaneously.

Beneath the vaginal unit.

Laterally to the pharynx (deep to the parotid gland).

Around the submaxillary gland (filling the submaxillary triangle).

In the suprasternal notch.

In the act of swallowing, certain members of the visceral group of structures must be allowed to travel up and down through their normal range of no less than half an inch, moving freely on the vertebral group behind and the ensheathing mechanism in front. At the level of the hyoid bone, this unit gains purchase from the base of the skull through the styloid process and its associated muscles, whilst depression of the unit is brought about by means of the infra-hyoid muscles which pass downwards to be inserted into the posterior surface of the manubrium sterni, and in the case of the omo-hyoid the superior border of the scapula. Thus it is evident that these two "fan-like" groups of muscles passing upwards and downwards from the hyoid bone are physiologically inseparable from the visceral unit and may be expected to exhibit cohesion by fascial condensations. The reality and importance of such condensations are confirmed in the present work.

The great vessel unit has only expansile pulsatile motion and is devoid of longitudinal movement. In spite of this it has to send its branches to the mobile visceral group which, in relation to the rest of the neck, moves like a tendon in its sheath. In order that this may occur all these vessels (lingual, superior thyroid, etc.) have a tortuous course, the "slack" being found between the two physiological units.

The vaginal unit, besides being concerned in the more gross cervical movements, serves to bind the structures it encloses into a working whole. Posteriorly, the vertebral group moves in conjunction with it, and in this situation the two units find close cohesion.

Only two of the units (visceral and great vessel) pass into the thoracic mediastinum. The former is covered by the infrahyoid muscles, which, being

attached to the back of the sternum, provide two surfaces, that in front terminating at the sternum; the other passing down behind reaches to the bifurcation of the trachea.

With these general considerations in mind, an attempt was made to determine to what extent the general neck structure and the connective tissue might reasonably be relied upon to limit, conceal or influence the course of injected fluid.

INVESTIGATIONS

These were made in three ways:

A. Dissections.

B. Large serial sections.

C. Injection experiments.

A. Dissections

Although it was found to be true that a plane of fascia can be demonstrated in whatever plane the dissector chooses, it was not difficult to show, by dissecting from a variety of both orthodox and unorthodox directions, that the cervical connective tissue is by no means of uniform density but is, in fact, a well-differentiated tissue exhibiting condensations of varying strength and containing areolar and fat-filled "spaces". These not only accorded with the general plan above mentioned, but were influential in directing and limiting fluid in the injection experiments.

The following observations were made from dissections:

(*a*) *Vaginal condensation*

There exists, commonly described as the superficial layer of deep fascia, a condensation encircling the neck like a tube. It embraces the trapezius and sternomastoid muscles. Towards the midline anteriorly, it is thin and is separated from the visceral unit by fatless areolar tissue. In the suprasternal notch is a pocket of fat enclosed within this ensheathing layer, the latter splitting to be attached to the anterior and posterior lips of the sternum (space of Burns). Followed backwards, the vaginal condensation, as it encloses the trapezius, fuses firmly with the condensation about the vertebral unit so that the two are not easily separated. Upwards, after passing the hyoid bone to which it is firmly fixed, it roofs in the submaxillary triangle and is attached to the lower edge of the mandible. Further back, however, it passes up to form a firm covering for the parotid gland but is only loosely attached to the zygomatic arch. It then passes on to cover the temporal muscle and ultimately gains the temporal ridge. In its upward course the vaginal unit is tucked up under the lower border of the parotid and here exhibits a special thickening between the styloid process and the mandible (stylo-mandibular ligament), about which the jaw moves. This condensation separates the parotid gland from the submaxillary triangle, and when followed still farther upward on the deep surface of the former rapidly loses its strength and becomes

so thin that it barely separates the parotid from the lateral pharyngeal fat pocket immediately deep to it. Apart from this large fat deposit, this salivary gland, unlike its submaxillary neighbour, has no fat or loose tissue around it, its fascial condensation being closely adherent to it. On its surface are situated the pre-auricular lymph nodes.

(*b*) *Vertebral condensation*

Surrounding the vertebral unit is a well-marked, but not particularly tough connective tissue condensation which is closely applied to it and penetrates between all its interstices so that its component parts are not easily torn apart. Postero-laterally it is fused with that part of the vaginal condensation which reinforces the deep surface of trapezius. More anteriorly it invests the scalene muscles and the brachial plexus and, traced downwards, it fuses with a condensation about the subclavian vessels to roof in securely the pleural apex.

The visceral unit is provided with its own condensation, and between the latter and the vertebral counterpart the connective tissue is areolar in nature and free from fat. This allows of visceral mobility. Running parallel with and three-quarters of an inch from the midline and extending from skull to thorax on either side are condensations of fascia reaching between the two units; so that, in effect, the retrovisceral (prevertebral) "space" is rectangular in section, its lateral boundaries being very short and serving to separate the space from the subvaginal region (i.e. the region deep to the vaginal unit). This retrovisceral space is a little constricted at the hyoid level and certainly reaches to the level of the arch of the aorta, although its lateral limits are here uncertain.

(*c*) *Visceral condensation*

Like the vertebral unit, this group of structures has a connective tissue condensation surrounding it and penetrating between its component parts. Unlike that unit where the intermuscular fascia is dense, however, the intervisceral fascia is much looser in its arrangement and the oesophagus is fairly easily separated from the trachea except in the region of the thyroid gland where this does not seem to apply. The fascia about the latter is strong.

As already stated, the styloid apparatus and the infrahyoid muscles constitute fan-like accessory parts to the visceral unit and these are both provided with condensations. In the case of the infrahyoid group, the omohyoid in its course laterally pulls out a fairly definite triangular condensation. Thus an areolar-filled space is left between this condensation and the vaginal unit, which is limited below by the origin of the infrahyoid muscles from the sternum and by the condensation around the subclavian vessels, and above and laterally by the omohyoid. Behind this lower fan and in front of the viscera is a further space which appears to reach down through the mediastinum to the pericardium.

The superior fan-like group effectively shuts off the upper from the lower

lateral visceral region. The upper division, here referred to as the lateral pharyngeal space,[1] is filled with fat and is deeply placed, being concealed from without by the parotid gland which is securely wedged in between the mastoid and mandible (Plate IV). Since, in spite of the fact that this space has received but little notice in this country, Mosher, Ford and others have shown clinically that it is the key position to difficult upper cervical suppurations, a full description of it is thought to be justified. It will be seen from the following account and especially from Plate IV how, on the one hand, it is intimately related to such potent sources of infection as the tonsil, pharynx, submaxillary triangle and mastoid process, and on the other it is difficult of surgical access.

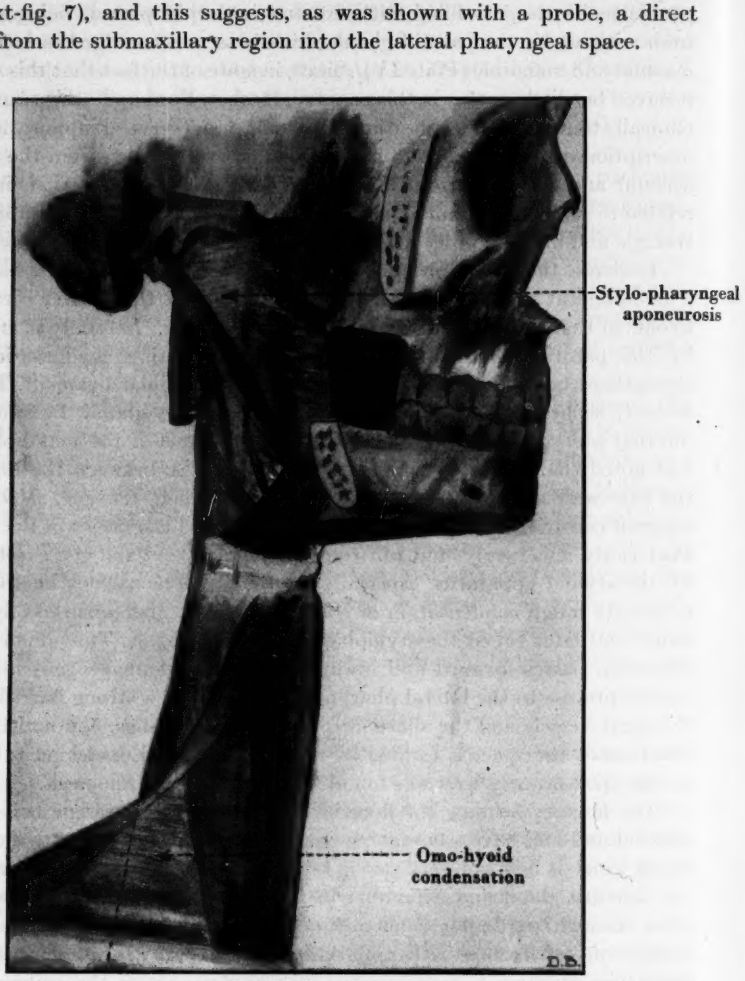
In shape, the lateral pharyngeal space is pyramidal, having its base nearly 1 in. square at the base of the skull and its apex at the greater cornu of the hyoid, so that its vertical axis measures about 1½ in. Laterally it is bounded by the parotid gland with its thin internal limiting condensation which strengthens below to form the powerful stylomandibular ligament. The latter securely separates the parotid from the submaxillary gland. In front are the internal pterygoid muscle and the ascending ramus of the mandible, and it was noted that it was not difficult to push a probe between the former and the side wall of the pharynx into the submaxillary triangle. Medially the superior constrictor separates it from the tonsil and the cavity of the pharynx. Posteriorly it is firmly shut off from the jugular vein and the carotid artery by the styloid apparatus, especially by the stylopharyngeus muscle and an extremely tough condensation of connective tissue first remarked by Zuckerkandl and later called the stylopharyngeal aponeurosis. This important condensation passes forward and inward from the stylopharyngeus muscle and styloid process to the lateral pharyngeal wall, and is a strong barrier between the great vessels and the space in question (see text-figs. 5, 6 and Plate IV). The base of the space is formed by the skull and the Eustachian tube, whilst at the apex no easy way was found leading down into the neck.

The muscles forming the floor of the submaxillary triangle belong to the visceral unit and have a fascial covering. Between this and the roof of vaginal fascia which is here not very strong is a quantity of fat in which are embedded the salivary gland and a number of lymph nodes. The gland was found to have its own particular condensation, and unlike the parotid it could be shelled out of its bed with remarkable ease. From the present standpoint there thus appear to be two potential submaxillary spaces, the one immediately surrounding the gland and the other outside its capsule. It is thus evident that an infection having its origin in the jaw or in a lymph node will not necessarily involve the gland and the latter should therefore not be incised indiscriminately.

As the gland turns round the posterior border of the mylohyoid muscle,

[1] The lateral pharyngeal space, although not actually labelled as such, can be studied in *An Atlas illustrating the Topographical Anatomy of the Neck, Thorax, Abdomen and Pelvis*, by Johnson Symington.

its elbow juts in between the internal pterygoid and the lateral pharyngeal wall (text-fig. 7), and this suggests, as was shown with a probe, a direct passage from the submaxillary region into the lateral pharyngeal space.



Text-fig. 5. The visceral unit showing the stylopharyngeal and omohyoid condensations.

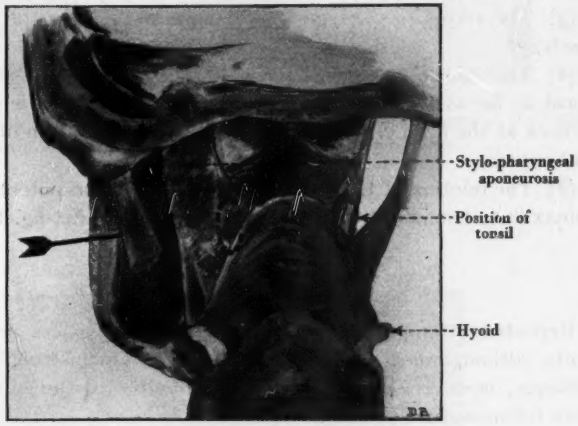
(*d*) *Condensation about vessels*

Definite condensations occur around all the cervical vessels. The term "carotid sheath", although familiar, has, however, but a limited application. Dissections seem to show that there is a common condensation enveloping the carotid artery and jugular vein but that each has its separate well-defined compartment, the venous part being less firmly adherent to its vessel. This

part we might properly call the jugular sheath. In the same way the lesser vessels are provided with sheaths, and the fact that these communicate with that of the main trunk is shown in the injection experiments.

It is also noteworthy that the internal jugular vein has some seven cervical tributaries, whereas none of their arterial counterparts come from the common or internal carotid arteries.

Lastly, the stylopharyngeal aponeurosis above described, being perhaps the strongest fascia in the neck, securely seals off the internal carotid artery and internal jugular vein from the lateral pharyngeal space in front of them; and further, the apex of this space does not lead into the sheath of either vessel as suggested in Beck's trifolium theory.



Text-fig. 6. The position of the lateral pharyngeal space in relation to the stylopharyngeal aponeurosis.

Inferences from these findings in relation to jugular vein thrombosis are drawn below.

B. Large serial sections

As a confirmation of the above findings, serial sections were made of the whole neck in a variety of planes. For this purpose, full-time foetuses were used. Celloidin embedding was found to be preferable to paraffin, since the former technique prevented the large sections from breaking up during the cutting and staining.

From these sections, some photographs of which are here included, the following points were noted:

(1) That the cervical connective tissue is a continuous system having condensations, fatty and areolar areas. Sections of a 3 months' foetal neck show that this differentiation is well seen even then.

(2) The size and reality of the lateral pharyngeal space is evident (Plate IV and text-fig. 7), and its relation to the following structures is well seen:

(*a*) Tonsil and lateral pharyngeal wall.

(*b*) Parotid gland which separates it from the skin.

(*c*) The great vessels in their condensations, separated from the space by the stylopharyngeal fascia.

(*d*) The submaxillary gland whose elbow projects backwards between the internal pterygoid and the hyoglossus and comes into relation with the space (text-fig. 7 only).

(*e*) The ramus of the mandible and the last molar tooth.

(*f*) The digastric muscle (corresponding a little higher up with the position of the mastoid process).

(*g*) The retrovisceral region (also note the relation of this to the great vessels).

(3) That the retrovisceral space is traceable from the base of the skull almost as far as the diaphragm (text-fig. 8). Text-fig. 9 is a section through the neck at the level of the larynx and shows the relation of this space to the great vessels.

(4) The relation of the submaxillary gland to the potential pathway from submaxillary triangle to lateral pharyngeal space (text-fig. 10).

C. Injection experiments

Repeated attempts were made to inject the spaces described, and the results, although not uniformly satisfactory, are instructive. No injection technique, however, can be strictly comparable to the spreading pus of an active inflammatory process.

Injection technique

For the purpose of this work it was necessary that the injection fluid should have the following properties:

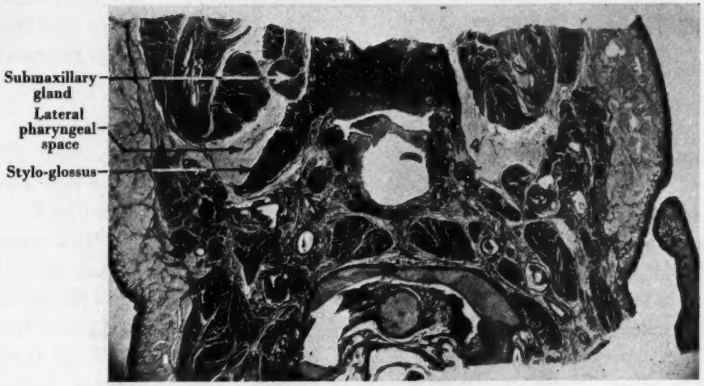
(1) That it should be sufficiently opaque to X-rays to ensure that a thin layer of it was visible under the screen and in films.

(2) That it should set solid after injection and should be of a colour easily distinguishable from the tissues, so that it could be dissected out afterwards and its position confirmed.

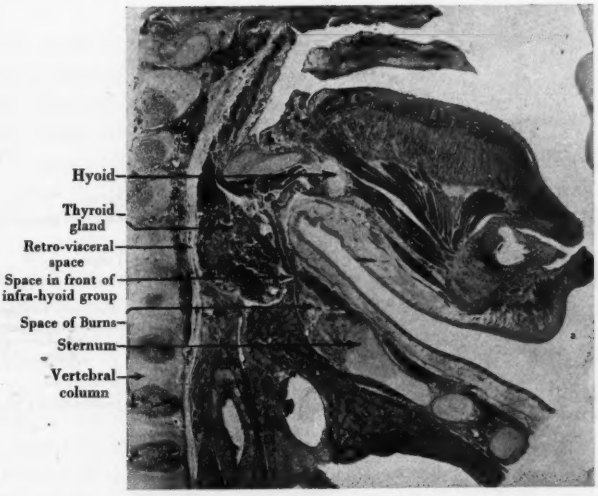
(3) That it should not set too quickly and block the needle. The fluid found to be most suitable was:

Barium sulphate	100 gm.
Gelatine	100 gm.
Water	1000 c.c.

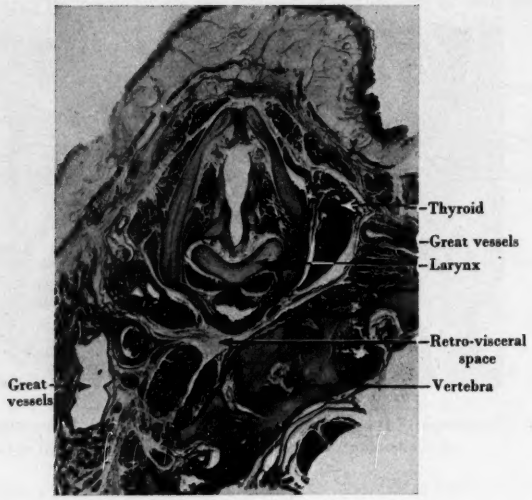
heated to 120° and thoroughly mixed. The subject for injection is also well soaked for half an hour in water at the same temperature. On allowing to cool after injection, firm setting occurs within 48 hours.



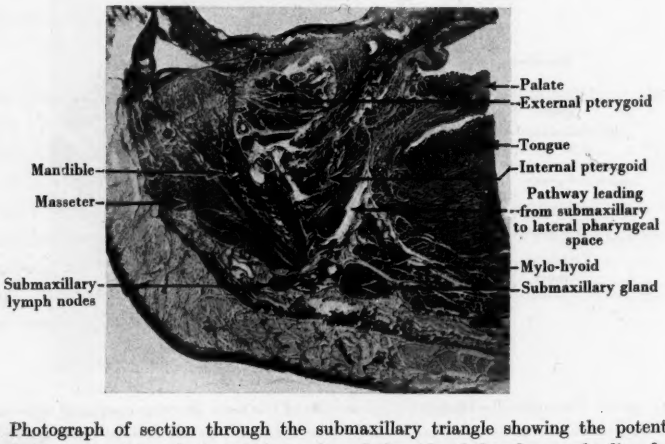
Text-fig. 7. Photograph of transverse section of the neck showing the relation of the "elbow" of the submaxillary gland to the lateral pharyngeal space.



Text-fig. 8. Photograph of longitudinal section of the neck showing extent of retrovisceral space. The space of Burns can also be clearly seen as well as the areolar filled space between the vaginal unit and the infrahyoid fan of the visceral unit.



Text-fig. 9. Photograph of transverse section of the neck at the level of the larynx showing the relation of the retrovisceral space to the great vessels (specimen somewhat twisted).



Text-fig. 10. Photograph of section through the submaxillary triangle showing the potential pathway between the internal pterygoid muscle and the side of the pharynx leading from the triangle to the lateral pharyngeal space.

Sufficient adult bodies being difficult to obtain, fresh full-term foetuses were used, the injections being made with a 20 c.c. record syringe. The injections were controlled under the X-ray screen and stereoscopic films were taken at frequent intervals.

Retrovisceral space

Injections of this space were made by removing the vault of the skull and the brain and injecting vertically downwards through the occipito-sphenoidal junction in the midline, the needle being controlled by a finger in the mouth.

The fluid was seen to track downwards between the vertebral and visceral units and to have a definite tendency to be obstructed in its lateral spread to the subvaginal space. Also it did not pass easily downwards into the posterior mediastinum below the level of the third thoracic vertebra. The reason for the latter fact is not explained, since in longitudinal sections the path appears free (text-fig. 8), but the limitation of its lateral spread was due to the laterally placed viscero-vertebral condensations already described. Only when considerable pressure was used could the fluid be made to penetrate this barrier and to burst into the subvaginal space. It was noted that under this amount of pressure, fluid also tracked backwards to some extent, along the course of the intertransverse branches of the vertebral vessels (Plate I, fig. 1). Fluid could not be made to burst into the lateral pharyngeal space.

Lateral pharyngeal space

Injections of this space were made in three ways:

(1) Through the parotid gland from the side.

(2) Through the base of the skull from above.

(3) Indirectly via the submaxillary space.

The space filled very easily and its size and shape are well seen in Plate I, figs. 2 and 3. In spite of the fact that considerable force was used after the space was filled, it was found difficult to make the fluid burst out of it, although it did so on two occasions; once into the submaxillary space (Plate I, fig. 4), and once into the parotid gland (Plate II, fig. 1). When it was approached by injecting the submaxillary space, however, the fluid tracked from the latter into it without much difficulty (Plate II, figs. 1, 2).

When the space was distended it was noted that the lateral pharyngeal wall was bulged inwards, carrying with it the tonsil on the anterior part of the bulge. At the same time there was a diffuse swelling of the side of the face caused by the lateral displacement of the parotid gland.

In none of the injection experiments was the stylopharyngeal aponeurosis penetrated. In all cases it had protected the great vessels wedged in behind it, a finding which suggests that the thrombo-phlebitis of the jugular vein, which is not uncommonly found in cases of lateral pharyngeal space suppuration and which Mosher and Ford believed to be due to direct infection, may have to be explained in another way (*vide infra*).

Injections of this space illustrate, in addition, the following important points:

(*a*) The depth of the space from the surface and its proximity to the base of the skull.

(*b*) Its accessibility from the pharynx.

The submaxillary space

In spite of the results of dissection it was not found possible by injections to demonstrate the gland sheath separately from the whole space. This may have been due to the difficulty of putting the point of the needle within the gland in such a way that leakage did not occur.

Injected fluid tracked in the following directions as pressure was increased and this usually occurred in the order stated:

(1) Round the posterior border of the mylohyoid muscle to the floor of the mouth, where it produced a large bulge.

(2) Backwards from the region of the elbow of the gland between the internal pterygoid muscle and the side of the pharynx, directly into the lateral pharyngeal space which it filled, but from which it did not escape.

(3) Downwards and backwards inside the condensation about the common facial vein and its tributaries or even inside that of the lingual vein. By this route it not infrequently entered the condensation about the internal jugular vein and extended down and even up it throughout the length of the neck (Plate II, fig. 2). In no case did the fluid enter the lumen of the vein, but in one instance (Plate II, fig. 3) it ultimately burst from the jugular sheath into the subvaginal space.

These three types of fluid tracking are illustrated to varying extents in Plate II, figs. 2 and 3.

Jugular sheath

Direct injection of fluid outside the internal jugular vein, but within its condensation was not as difficult as was anticipated. It was carried out from below after removal of the major part of the thorax and was strikingly successful on one occasion (Plate II, fig. 4). Unfortunately, after these films were taken, the needle inadvertently entered the vein and prevented further injection.

Parotid space

Injections were made into the parotid gland beneath its strong superficial condensation. The gland itself rapidly became thoroughly infiltrated, and as pressure was increased the fluid began to track from it as follows:

(1) Upwards superficial to the zygomatic arch towards the temporal fossa (Plate III, fig. 1). This upward extension was never very large and remained near the surface.

(2) Inwards into the lateral pharyngeal space only when the pressure was considerable.

(3) Downwards within the condensations of the posterior facial and superficial temporal veins towards the common facial and external jugular veins respectively. This perivenous tracking did not occur here with the same readiness as it did in the case of the submaxillary space, but it can be seen commencing in Plate III, fig. 1.

Space of Burns

This space was injected quite easily (Plate III, fig. 2). As pressure increased the fluid entered the subcutaneous tissues.

Subvaginal space

This space, containing, as it does, large numbers of lymphatic nodes, is more liable to infection than any other. It filled readily when injected (Plate III, figs. 3, 4), and was seen to be most marked in the posterior triangle and beneath the sternomastoid muscle. Fluid did not pass far forward into the anterior triangle, nor far backward under the trapezius. Moreover, there seemed to be little tendency for it to enter the retrovisceral space or previsceral region. In Plate III, fig. 4, in which case considerable pressure was used, it will be seen that the fluid has still not crossed the midline, although it has extended throughout the length of the side of the neck and has even commenced to leak into the thorax behind the sternum. This was the only instance in which there was any sign of leakage into the thorax when injections were made beneath the sternomastoid, and this may have been due to damage caused by the injecting needle. In no instance did fluid find its way into the parotid, submaxillary or lateral pharyngeal spaces, nor did it get into the jugular sheath.

The spaces in front of the visceral unit

Although dissections and sections suggested that in this situation two independent spaces, one in front of and the other behind the infrahyoid muscles, exist, they were found difficult to demonstrate by experimental injections. This, however, may have been due to the considerable difficulty of fixing the point of the needle in the proper places while the injections were in progress.

Plate III, figs. 5, 6, show that the space in front of the muscles reaches from hyoid to manubrium sterni, and although no satisfactory pictures were obtained of the space behind them, the injected fluid seemed to find its way in amongst the viscera and particularly around the trachea, as well as downwards nearly to the pericardium.

SUMMARY AND CONCLUSIONS

The following anatomical conclusions are drawn as a result of certain dissections, serial sections and radio-opaque injections of the neck.

(1) The cervical connective tissue has an important influence in directing and limiting the spread of injected fluid.

(2) The retrovisceral space reaches from skull well down into the posterior mediastinum, and is limited laterally by condensations of connective tissue. Above the level of the hyoid, fluid will not readily burst from it laterally and it does not communicate with the lateral pharyngeal space. It has no direct relationship to the tonsil but contains lymph nodes draining the nasopharynx. Below the level of the hyoid its lateral boundaries are less strong and fluid may burst from it into the subvaginal space.

(3) Apart from the retrovisceral space there are three other important potential spaces above the hyoid level: submaxillary, parotid and lateral pharyngeal. Whilst pus within the second, although an uncommon occurrence, always involves the salivary gland, pus within the first does not necessarily do so, and the space should be opened with caution.

Pus in either of the last two regions tends to be prevented from pointing on the surface by the vaginal condensation, but from the parotid space it may travel inwards into the more deeply placed lateral pharyngeal space.

(4) The lateral pharyngeal space itself is a dangerously concealed reservoir and may become infected as above stated or directly from the tonsil, pharynx or mastoid process. When distended the pharynx is bulged inwards with the tonsil on the front of the bulge, and this fact may assist in an otherwise difficult diagnosis. The space is not easy of surgical access, but according to Mosher and others it is best approached from without.

(5) Internal jugular vein thrombosis is not an uncommon happening in a neglected case of deep cervical suppuration. Its occurrence has hitherto been explained by supposing that the lateral pharyngeal space was first infected, and that from there pus reached the "carotid sheath" by direct extension either through the apex of the space or through the stylopharyngeal aponeurosis. In this work it has been found that both these routes to the great vessels are highly resistant to the passage of injected fluid. On the other hand, fluid from the upper neck spaces will reach the *jugular sheath* with moderate ease via the sheaths of that vein's tributaries. If this is the true explanation of the thrombosis, it follows that its occurrence or extension is best prevented by ligature, not only of the veins leaving the infected area, but also of their sheaths. It is notable that arterial sheaths could not be injected in the same way as their venous counterparts.

(6) The neck is enclosed within a musculofascial tube—the vaginal unit—which is apt to mask inflammatory lesions and hinder their pointing on the surface. The subvaginal spaces on either side do not communicate. They are deepest beneath the sternomastoid muscles and contain many lymph nodes which constitute their principal source of sepsis, as well as the great vessel units. The three great suprahyoid spaces do not open into them, but they are not very strongly shut off from the retrovisceral compartment. Pus passing from the latter into them does so behind the great vessel unit.

The previsceral spaces, with the exception of the space of Burns, have not been fully investigated, but it would appear that there are two principal

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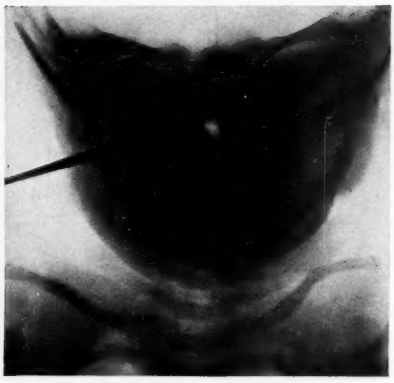

Fig. 2.

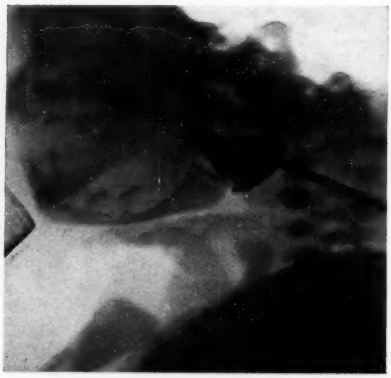

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Fig. 4.

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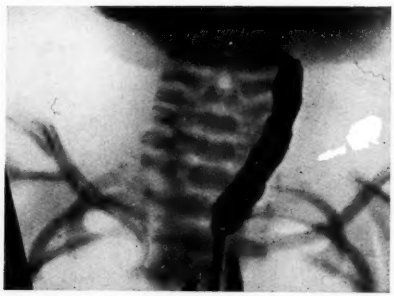

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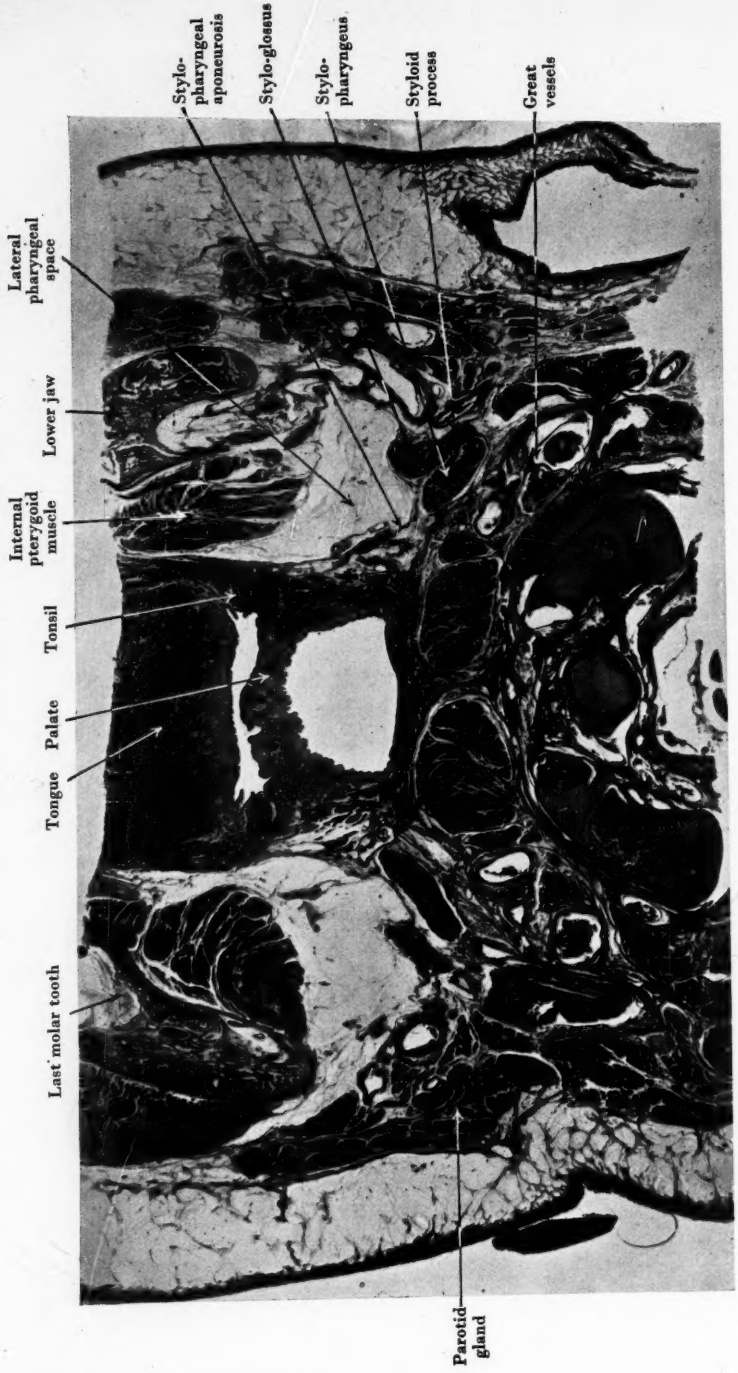

BARLOW—The Surgical Anatomy of the Neck

potential compartments separated by the infrahyoid fan-like group of muscles. Only the posterior compartment reaches more than an inch into the thoracic opening and they do not communicate.

This work was carried out at University College Hospital Medical School and in the Anatomy Department of University College. I am much indebted to Prof. C. C. Choyce, and to Prof. Sir Grafton Elliot-Smith for so generously providing me with the materials and facilities, to Dr A. Durward for many helpful suggestions and to Mr Melville for his assistance with the X-ray work.

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EXPLANATION OF PLATES I–IV

PLATE I

Fig. 1. Lateral view of injected retro-visceral space, showing its extent from base of skull, well down into the thorax. Backward extensions between the transverse processes are also visible.
Fig. 2. Antero-posterior view of injected lateral pharyngeal space, showing its size and shape and its relation to the base of the skull.
Fig. 3. Lateral view of lateral pharyngeal space.
Fig. 4. Lateral view of injected lateral pharyngeal space showing forward extension into the submaxillary space.

PLATE II

Fig. 1. Oblique view of injected lateral pharyngeal space showing lateral extension into the parotid gland.
Fig. 2. Lateral view of similar case showing the triple extension. In this instance, the sheaths of the lingual, common facial and internal jugular veins are affected. That of the jugular vein is outlined as far down as the thoracic opening and there is also some upward extension.
Fig. 3. Lateral view of an injection into the submaxillary space, considerable pressure being used. It is seen that the fluid has burst from the jugular sheath into the subvaginal space.
Fig. 4. Antero-posterior view of the jugular sheath injected from below.

PLATE III

Fig. 1. Antero-posterior view of injected parotid space showing the upward extension superficial to the zygomatic arch. At the lower pole of the injected area, small extensions may be seen which were actually in the sheaths of the temporal and posterior facial veins.
Fig. 2. "Space of Burns" injected.
Fig. 3. Lateral view of injected subvaginal space.
Fig. 4. Antero-posterior view of injected subvaginal space.
Fig. 5. Lateral view of the anterior pre-visceral space.
Fig. 6. Antero-posterior view of same.

PLATE IV

Photograph of transverse section of the neck at the level of the tonsil to show the relations of the lateral pharyngeal space.

CONGENITAL UROGENITAL ANOMALIES IN RATS INCLUDING UNILATERAL RENAL AGENESIA[1]

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Department of Midwifery, University of Edinburgh

Structural abnormalities which have been observed in certain rats of a stock bred primarily for experimental purposes appear to be worth recording, not only on account of their rarity, but also because of the existence of a close relationship between the affected individuals, and of the fact that similar anomalies have been encountered in man (Collins, 1932; Campbell, 1928; Eisendrath, 1924; and others).

The stock consists of pure albino rats (Wistar) and a cross between these and a "hooded" variety in both of which abnormalities are rarely found, although microphthalmia has occurred in the pure-bred stock and in the crosses. There is no known record of the existence of the abnormalities to be described either in mice or in rats. The stock has been injected with endocrine preparations over a period of years and more especially pregnant animals have been used; it has been impossible, however, to find any direct association between such treatment and the anomalies observed. The genealogical table reproduced suggests that the character is present as a recessive with various modifications of its expression, and its association with a microphthalmic ancestry diluted by outcrosses points to a possible connection with the factor for microphthalmia, such as has been cited by Collins (1932) in the list of anomalies associated with renal agenesia collated by him from the literature bearing on the human subject. From the descriptions which follow it would seem that, whereas unilateral renal agenesia in the rats observed was always accompanied by genital maldevelopment, the latter can occur in related individuals without renal defects.

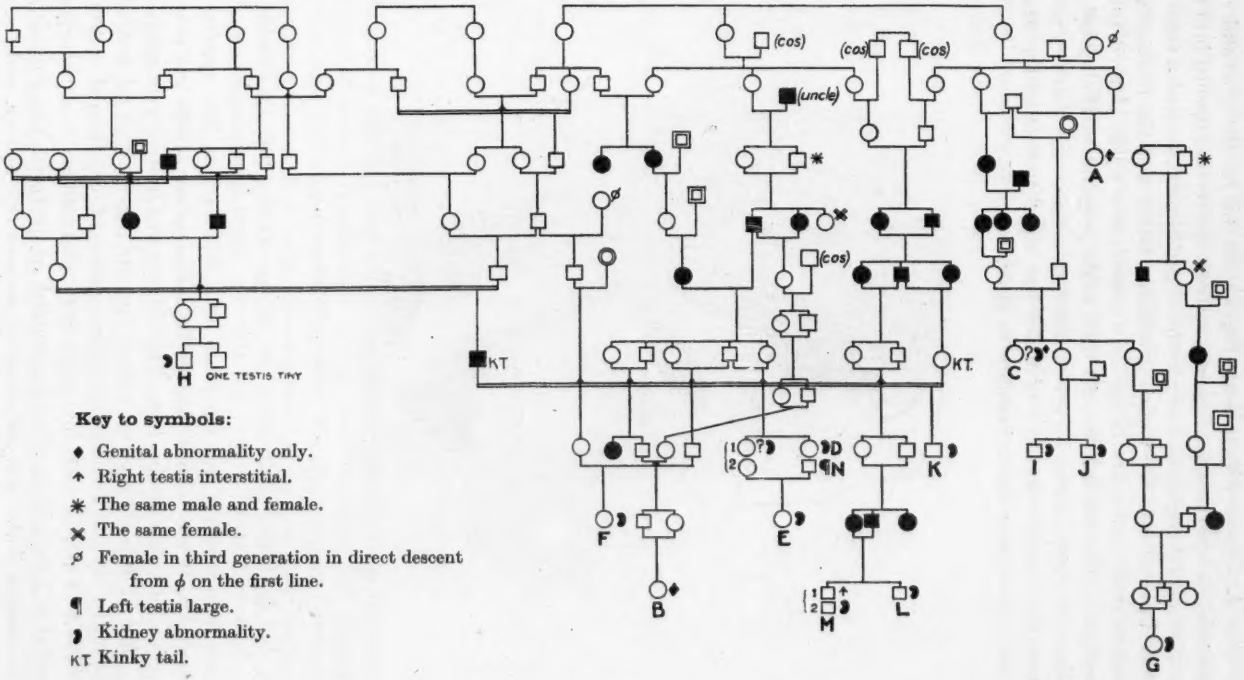
DESCRIPTION OF THE ABNORMALITIES

It will be found that towards the end of this investigation the abnormalities are more fully recorded, as it was seen that the earlier observations were not sufficiently comprehensive. In the description which follows, the rats bear the initials given to them in the genealogical table.

[1] The expenses of this investigation were largely defrayed by grants from the Medical Research Council and the Carnegie Trust for the Universities of Scotland.

Pedigree of rats having renal and genital anomalies

A circle denotes a female; a square denotes a male. Outcrosses (i.e. a different strain of rats) are represented by a circle within a circle and a square within a square. Solid squares and circles denote microphthalmics.



Key to symbols:

- ♦ Genital abnormality only.
- ↑ Right testis interstitial.
- ✳ The same male and female.
- ✕ The same female.
- ⌀ Female in third generation in direct descent from ϕ on the first line.
- ¶ Left testis large.
- ❩ Kidney abnormality.
- KT Kinky tail.

Rat A. Female with a cloaca (fig. 1). In this rat the anus and vagina did not exist as separate openings but, instead, the rectum opened into the dorsal portion of the vagina and within the vaginal lumen, so that a smearing wire, when inserted into the vagina penetrated either into the rectum or into the vagina proper. Smears taken for a period of 4 weeks showed that normal oestrous cycles existed; the rat died while pregnant at the age of 4 months when the right uterine horn was found to contain four foetuses and the left horn five, the development of which was equal to that of the 22nd day or term. Death was due to inability to litter.

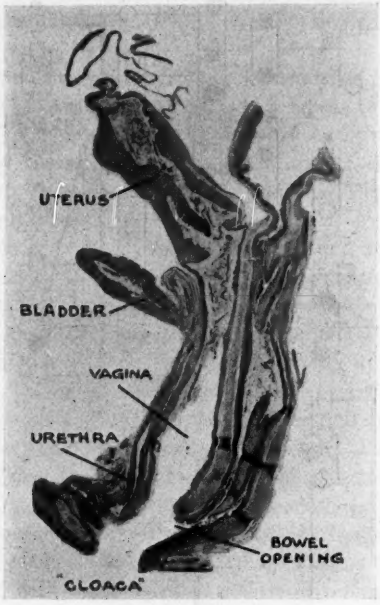


Fig. 1. Female rat A.

Rat B. Female with abnormal vaginal opening (fig. 2). This rat appeared to have no vaginal orifice, but this was found immediately anterior to the anus from which it was separated by a thin septum. The opening was very small, and it was with difficulty that a fine suture needle was passed up the vagina into each uterine horn. The kidneys and ureters were normal.

Rat C. Female with no vaginal opening (fig. 3). This rat was killed when 5 months old and immediately after a course of injections of oestrone, during which 1·44 mg. or 7500 int. units were administered subcutaneously over a period of 4 days in an effort to establish an aperture. The normal position of the vaginal orifice was indicated by an area free of hair, and the occluding

membrane appeared to be of a uniform thickness. The distance from the anus to the centre of this area measured 11·75 mm. At necropsy the right uterine horn was found to be greatly distended with fluid and was 8 mm. wide × 45 mm. long. A sharp constriction at the cervix marked its entrance into a distended and closed canal 14 mm. in diameter and 42 mm. long, which proved to be the vagina. This did not connect with the natural site of the vaginal orifice but followed the course of the rectum along its entire extent although it did not open at the anus.[1] The left uterine cornu was represented by a small spherical sac situated immediately below the ovary; it measured only 11 × 8 mm. and was filled with fluid.

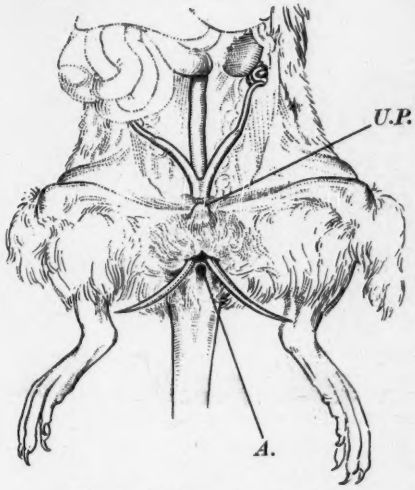


Fig. 2. Female rat B. *A.* anus, *U.P.* urethral prominence.

Fig. 3. Female rat C.

It is likely from the data about to be presented demonstrating the association between defects in development of the genital system and kidney anomalies, that rat C had a hypoplastic kidney on the defective side; however, this was the first animal in this series encountered with such an abnormality, and as no such association had at that time been observed, the existence of both kidneys was not ascertained.

Absence or hypoplasia of the left kidney

In the rats about to be described the absence or hypoplastic development of a kidney has always been found in association with genital abnormalities on the same side of the body, and it has always been the left side which has been affected.

[1] A similar defect has been observed in a mouse, but both uterine cornua were normally developed.

Rats D and F. Female rats in which neither kidney, ureter nor renal blood vessels were to be found on the left side (fig. 4). The adrenal gland and the ovary were present and the latter was normally situated, but no left uterine cornu existed. The right side of both rats was, in all respects, normal.

A female in the same litter as rat D was found to have no left cornu; this rat was unfortunately destroyed without complete examination.

Rat E. Female rat with hypoplastic left kidney (fig. 5). On the left side the place of the kidney was taken by a hard, yellowish body the size of a pea, which, on histological examination, proved to be a cystic, hypoplastic kidney. The renal artery and vein on this side were normal, but no ureter was found. Below the defective kidney were a normal ovary and a portion of uterus about

Fig. 4. Female rats D and F.

Fig. 5. Female rat E.

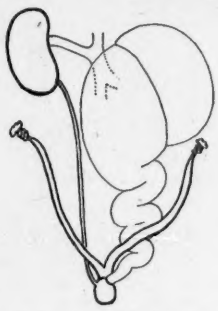


Fig. 6. Female rat G.

10 mm. long, having a blind lower end. The right side was normal. Littermates killed at the same time as rat E exhibited no abnormality. (See male rat N, i.e. father.)

Rat G. Female rat with hydronephrotic kidney and hydroureter on the left side (fig. 6). This female was killed when 42 days old; at that time she weighed only 15 gm. as compared with females in the same litter which weighed 90 and 92 gm. A litter-mate of her mother had a similar tiny female in her litter, but this was eaten on the day that rat G was killed. It is possible that a similar condition existed.

On the left side in place of the kidney there was a large translucent, hydronephrotic sac; the renal pelvis was greatly enlarged, also the ureter which was much kinked on itself. Under the dissecting microscope it was found that the left ureter was obstructed at its junction with the bladder and terminated at the site of its normal opening in a cystic dilatation projecting into the base of the bladder. The condition was apparently one of stenosis of the lower end of the ureter at its entry into the bladder, with subsequent distension of the occluding portion and also of the renal tract proximal to the barrier. Microscopic examination did not reveal any clue as to whether the cause lay in the ureter, the bladder wall or the bladder mucosa. On the embryological data,

however, it is evident that a secondary occlusion of the ureter must have occurred. As the lumen of the ureter is an outgrowth from the existing archinephric (i.e. mesonephric) duct, there must once have been a clear opening. If there had been no archinephric duct, there could have been no mesonephros, but in the two male rats exhibiting a similar abnormality (rats L and M) the epididymis, itself derived from the mesonephros, is well marked.

The ovary and uterus on the defective side were normal, as also were the organs on the right side.

Rat H. Male rat with no left kidney (fig. 7). On the left side there was no trace of kidney or ureter. Midway between the normal renal site and the pelvic girdle lay a small testis only 10 mm. long; the epididymis was below

Fig. 7. Male rat H.

Fig. 8. Male rat I.

this and not contiguous with the testis, and from it there arose three strands which appeared to bury themselves in the abdominal wall; the gubernaculum connecting with the scrotal sac was 55 mm. long. The seminal vesicle on the left side was underdeveloped, measuring only $7{\cdot}5 \times 6{\cdot}5$ mm. and weighing 100 mg. (cf. $28 \times 11{\cdot}5$ mm. and 1·0 gm.—normal).

The right kidney was greatly enlarged and weighed 2·7 gm. (cf. normal litter-mate's kidney 1·6 gm.). The right testis and seminal vesicle were normal. There was much diffuse chromaffin tissue on both sides. A male in the same litter was a typical unilateral cryptorchid with no kidney abnormality.

Rat I. Male rat with no left kidney (fig. 8). On the defective side no renal vessel and no ureter were found. The testis occupied a position a little below the normal site of the kidney and away from the midline; it weighed only 0·2 gm., while that on the right side weighed 0·85 gm. There was a complete absence of ducts on the left side, and no seminal vesicle.

The right kidney was greatly enlarged, as in rat H, and weighed 2·2 gm. (cf. 1·6 gm., the average weight of a kidney of an animal of the same body weight). The seminal vesicle was peculiar in that it consisted of two lobes, the one arising out of the centre of the other.

Rat J. Male rat with an atrophic left kidney (litter-mate of rat I) (fig. 9). A small atrophic mass of tubules about 5 mm. long lay in a pad of fat, and the whole mass was connected to the abdominal vessel by a pedicle of blood vessels which was twisted on itself twice. That these tubules were renal tissue was verified by section, but as these must evidently have been a remnant of the mesonephros there was no ureter. The testis on the same side was abdominal, very small, and lay 30 mm. below the "kidney"; it had neither attachments nor vas. The gubernaculum was represented by a thin fibrous

Fig. 9. Male rat J.

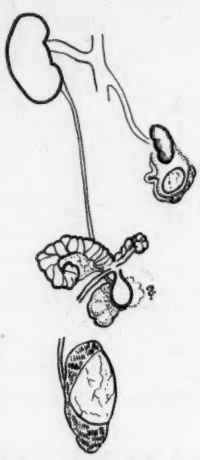


Fig. 10. Male rat K.

Fig. 11. Male rat L.

cord which disappeared into the inguinal canal. The seminal vesicle was small and, like other underdeveloped vesicles in this series, was quite translucent. The prostate on this side was also very tiny and semi-transparent.

The right side was normal.

Rat K. Male rat with cystic hypoplastic left kidney (fig. 10). On the defective left side a small, soft and amorphous kidney lay about 25 mm. below the normal position. The testis on this side was tiny and had not descended (male 3 months old). There was no connection with the seminal vesicle which was underdeveloped as in rat J, and passing from the small globus major a thin, fibrous strand connected with the outer side of the kidney, i.e. the side remote from the hilum.

Rat L. Male rat with polycystic left kidney (figs. 11 and 12); killed when 7 weeks old, along with its litter-mate in which the right testis was interstitial in position but in which there was no renal abnormality.

Rat L exhibited considerable urinary incontinence. At necropsy the left kidney, which was in the normal position, was found to be almost three times the size of the right kidney and was a mere sac of renal tissue, distended with fluid; the wall was normal in colour; the left ureter was much dilated. By the use of probes and with the aid of the dissecting microscope, it was found that, as in the case of female rat G and male rat M (his litter-mate), an obstruction apparently existed at the point at which the ureter was attached to the bladder at the left of the trigone, just above the neck (fig. 12). Cross-sections taken of the lower portion of the bladder and of the urethra demonstrated microscopically that the left ureter had no opening either into the bladder or directly into the urethra. The portion of bladder wall (?) obstructing the ureteral lumen shared in the general distension of that tract,

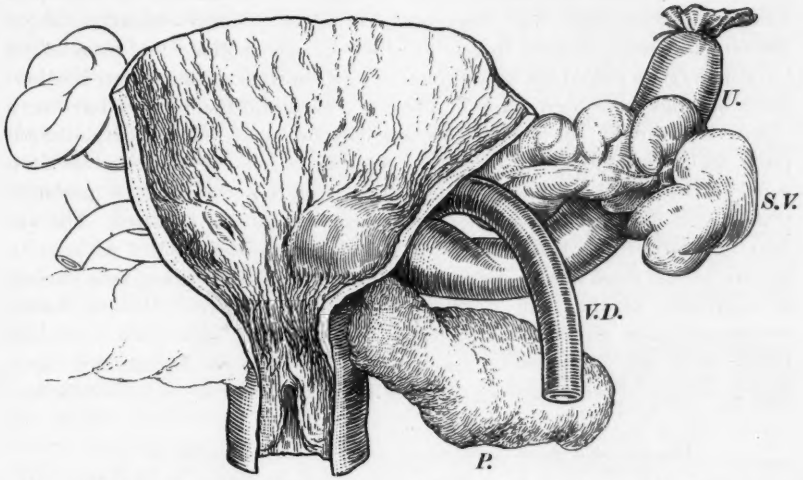


Fig. 12. Male rat L. *P.*=prostate; *V.D.*=vas deferens; *S.V.*=seminal vesicle; *U.*=ureter.

and this resulted in distortion of the neck of the bladder and consequent incontinence of urine. The testes, the vascular system of the seminal vesicles and vasa deferentia and of the bladder circuit were normal; the left lobe of the prostate was smaller than the right. The right side was normal.

Rat M. Born in a subsequent litter to the same parents as rat L. This male had shown signs of incontinence but to a less extent than its litter-mate, rat L. When killed at 5 weeks old, it was found to have a large, translucent, hydronephrotic kidney on the left side. Its appearance, and that of the ureter, which was also greatly distended and kinked, resembled in all respects the condition observed in female rat G already described (see fig. 6). The testes and other genital organs were normal.

Rat N. Kidneys normal but left testis was very much larger than the right

and exceeded any other testis encountered both in weight and in size. The measurements were as follows:

Left testis: 28 mm. long, weighing 3·0 gm.
Right testis: 20 mm. long, weighing 1·5 gm.

This male fathered female rat E already described.

Renal agenesia and hypoplasia as an inherited defect

The pedigree chart (p. 567) strongly suggests that the occurrence of the defect is not a random one. As the defect can rarely be detected externally in females, it is probable that instances of its occurrence in that sex have been overlooked; in males, however, all animals having a single descended testis or testicular underdevelopment have always been submitted to necropsy. Since the abnormality was observed to be a transmitted character, about 500 females closely related to rats in which a renal anomaly had been found have undergone palpation, laparotomy or post-mortem examination; brother-sister matings have been made in affected groups and their litters have been carefully inspected. In spite of this, the particulars are, of necessity, incomplete, and it is impossible to calculate with any degree of accuracy the incidence of the abnormalities; one can merely state that the defect is probably transmitted as a recessive and that several factors are involved. The appearance within the chain, of females having genital deformities without an accompanying renal defect, suggests that such abnormalities may be a variant in expression of the same developmental anomaly rather than a chance occurrence, more especially as similar abnormalities are mentioned by Collins (1932) in his list of congenital anomalies described in the literature as associated in cases of renal agenesia, a list which totals some seventy-five forms.

The morphological development of the structures involved

The varying degrees of renal defect would seem to be attributable to the stage at which the arrest of embryological development occurred, e.g. the entire absence of a kidney must belong to an earlier date than the hypoplastic kidneys of rats E, J and K, which, in turn, belong to an earlier stage than those of rat L and also rats G and M.

The regularity with which the left side only was affected is striking and suggests that an inequality exists in the rate of development of the two sides of the body. Gray (1930) has recorded that the right kidney of the common frog always develops more slowly than the left; this asymmetry is not observable after metamorphosis. Cases of lateral asymmetry described in birds (Crew, 1931; Crew and Lamy, 1935) and in man (Potter and Urwick, 1935; Bowman, 1935) have almost entirely related to limb size and to colour. When gynandromorphism was present this was associated with gonadic abnormality. Crew (1931) is of the opinion that the various expressions of asymmetry can

bear a single interpretation, viz. that "aberration in chromosome distribution is the cause of the regional expression of a recessive character" present in a heterozygote. He considers that the autosomal constitution is affected and explains the variations in manifestation as due to differences in time during development at which the loss of the particular autosome or its part occurred, the earlier the loss occurs the greater being the distribution of the recessive character.

In order that the earliest stages of the developmental idiosyncrasy might be observed, litter-mates of rats with renal agenesia were given to Dr Peter Gray (Zoology Department, Edinburgh University), when these were 8–12 days pregnant, for an embryological study of the abnormality, as it seemed possible that the defect would appear in closely related stock. The result of this investigation is not yet available.

The simultaneous occurrence of anomalies in both the genital and urinary systems in the affected animals is not surprising in view of the relation known to exist in the morphological development of the two systems. In those rats in which there is no trace of a urinary system (rats D, F and I) it is manifest that the defect dates to a stage prior to the development of the oviduct and archinephric duct and involves the non-development of either a mesonephros or pronephros. This is compatible with the absence of epididymis and ducts in rat I. In those rats in which the ureter is absent but renal tissue exists along with partial development of a duct system and of the seminal vesicle (rats H, J and K), the developmental arrest must have taken place after the formation of the mesonephros but prior to the formation of the metanephric bud, since the ureter and pelvis of the kidney are not found.

In female rats C and E it would seem that the arrest in the development of the oviduct occurred during the process of back growth of the inner end of the ostium abdominale tubae (Brambell, 1927). In describing the morphological development of the gonads of the mouse Brambell writes: "The Müllerian duct develops, as stated by Felix and de Winiwarter in the human species, as a back growth of the inner end of the ostium abdominale tubae. The similarity in structure of the primordium of the ostium abdominale tubae to the nephrostomes and its similar position in the urogenital ridge slightly anterior to them, suggests that they may be homologous. On this view the ostium abdominale tubae would be a nephrostome which grew and extended posteriorly to form the Müllerian duct." Thus these abnormalities add striking support to the theory, not universally accepted to-day, that the mammalian oviduct develops in a postero-anterior direction.

Since the mesonephros is already well established and the tubules formed anteriorly by the 9th day post coitus (Brambell, 1927), it is manifest that development can be arrested at a very early stage. The existence of some adverse environmental influence upon the developing embryo would have suggested itself had all the members of a litter been abnormal, but in only two instances were defective genitalia encountered in more than one member of a litter.

The occurrence of a male with renal maldevelopment in each of two successive litters born to the same parents suggests that an inherent defect existed in the parent cell, and the ovum itself may have been defective.

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ANATOMICAL NOTES

AN ADDITIONAL (THIRD AND SEPARATE) HEAD OF THE PRONATOR TERES MUSCLE

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From a perusal of the somewhat limited references at my disposal I am unable to say what the prevalence of the above condition is. Of the 200 subjects dissected in the Anatomy Department of the Rangoon Medical College during the last two years I have only come across one case. Gray mentions of course that the pronator teres

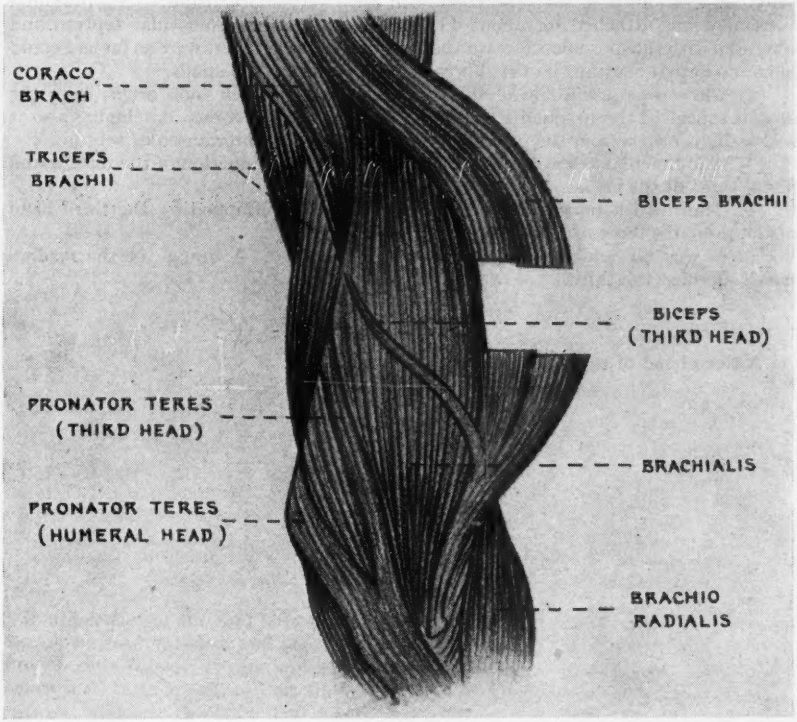

Diagram.

has an origin from the medial intermuscular septum between the muscle and the flexor carpi radialis and from the anti-brachial fascia. Quain mentions an extension of origin in a proximal direction to become attached to a supracondylar process or the ligament which connects this with the epicondyle. In these cases the course of the brachial artery is usually changed. In the text-books of Cunningham,

Spalteholtz and Berry I find no description of variations. Buchanan (5th edition) also mentions a third head arising from the internal intermuscular septum or from a supracondylar process. Quain dwells more on the ulnar head which is well marked in monotremes and absent in mammals, generally; is absent in the gibbon and gorilla but present in the orang and chimpanzee. In the latter (according to Sonntag) the condylar head is fused with the flexor carpi radialis, and the coronoid head with the flexor sublimis digitorum, and some fibres arise from the internal intermuscular septum.

The present case (see diagram)

The subject belonged to the financially depressed classes of Burmese in Rangoon. His occupation was probably that of a coolie.

The following conditions were found in this subject:

(1) A third head of the pronator teres muscle. This head was altogether separate from the usual superficial head of origin, so as to constitute a distinct and separate belly. It was joined to the superficial head for a very short distance. Its proximal extremity was attached for about 4 cm. to the medial intermuscular septum and was fused with the muscular fibres of the brachialis muscle. There were as far as I could judge no actual continuity of its fibres with those of the brachialis.

(2) There was a third head to the biceps brachii which took origin from the medial aspect of the brachialis below the insertion of the coraco brachialis; also it had a slight origin from the proximal part of the medial intermuscular septum.

(3) The brachial artery bifurcated at the level of the junction of the middle and distal third of the arm.

(4) The median nerve and brachial artery were overlapped by the third head of the pronator teres—but were not displaced.

There was no evidence of a supracondylar process. A branch of the median nerve supplied the third head of the pronator.

Remarks

A third head of the pronator teres is described.

ABNORMAL AZYGOS PLEURAL FOLD

By R. H. ROBBINS

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THE condition here described, although not a rare one, is sufficiently uncommon and sufficiently varied in extent to make its occurrence worth recording. The azygos vein, instead of being applied closely to the right mediastinal structures, stands clear away, and is enclosed in a crescentic fold of pleura drawn down from the upper end of the pleural cavity. The fold in the present case has a depth of about 2 in., and forms the lateral boundary of a definite recess. The fold subdivides the apical portion of the right lung, a part of which passes up medial to the vein and occupies the recess.

The condition appears to be brought about in the following way. At the 10-mm. stage of the embryo the lung bud lies within the pericardio-peritoneal channel, and is medial to the duct of Cuvier, which is situated in the lateral wall of the channel.

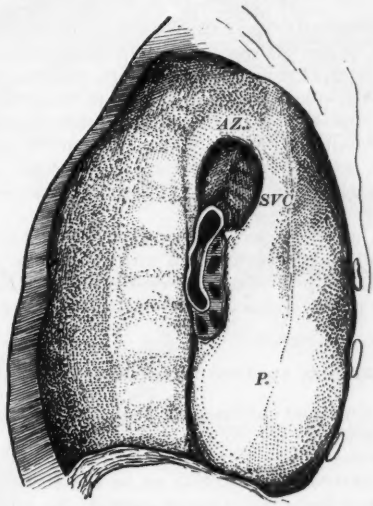

Fig. 1. Mediastinal wall of pleural sac, seen from the right. *AZ*, azygos vein; *SVC*, superior vena cava; *P*, pericardium, with indication of phrenic nerve.

The upper end of the bud is not clearly marked off at this stage, but very soon it begins to grow rapidly, and occupies the cranial end of the channel. The duct of Cuvier then (about 12 mm.) passes over the upper end of the bud, which slips beneath it, so to speak, so that the lung, as it burrows into the body wall, lies now entirely lateral to the duct. The unoccupied recess on the medial aspect of the duct becomes obliterated with the lowering of the general level of the pericardium and the drawing down of the terminal portion of the cardinal vein by the receding duct.

Should the cranial end of the bud grow too rapidly for the duct to pass completely over it, a portion of the lung is left occupying the recess on the medial aspect of the vein. When the proliferating tissue on the duct of Cuvier fuses subsequently with the medial wall of the passage, and shuts off the pericardial contents, the recess remains as a part of the pleural cavity, retained because it has been occupied by lung from the beginning.

NOTE ON AN UNUSUAL OPHTHALMIC ARTERY ASSOCIATED WITH OTHER ABNORMALITIES

By P. K. CHANMUGAM, M.R.C.S., L.R.C.P., L.M.S.
Department of Anatomy, Medical College, Colombo

An abnormal artery arising from the middle meningeal is of sufficiently rare occurrence to warrant a short note; still more so when associated with multiple abnormalities found elsewhere. The few previous cases were summed up by Meyer as long ago as 1886 (*vide* Quain, 1892).

The ophthalmic artery

In a female Singhalese body, aged 22 (No. 275 in the register of this department), the ophthalmic artery on the left side arose from the anterior division of the middle meningeal. It gave off a lacrimal branch before entering the orbit through the extreme outer end of the sphenoidal fissure. Within the orbit it passed deep to the ophthalmic vein and superior rectus muscle, to which it gave a branch, and then crossed, superior to the optic nerve, to the inner wall of the orbit. It then proceeded forwards between the internal rectus and superior oblique muscles giving off the anterior and posterior ethmoidal arteries. It finally terminated by dividing into the frontal and nasal arteries.

The nasal branch, after emerging from the internal tarsal ligament, took the place of the supra-orbital and anastomosed with the frontal and a branch from the superficial temporal. The supra-orbital artery itself was wanting.

The central artery of the retina and the ciliary arteries were given off from the internal carotid.

Arteries related to the temporal bone

In addition to the abnormal ophthalmic the middle meningeal gave off its normal petrosal branch directly opposite to the abnormal vessel. This took the normal course backwards into the aqueduct of Fallopius wherein it was found to anastomose with a vessel which proved on dissection to be the stylo-mastoid branch of the posterior auricular artery from the external carotid. No artery passed through the hollow of the stapes.

Other arterial abnormalities

(*a*) The right subclavian artery arose from the descending aorta and passed obliquely upward behind the oesophagus. This is pattern E of De Garis, Black and Riemenschneider (1933). The order of branches from the aortic arch was therefore right common carotid, left common carotid, left subclavian. The right vertebral was a branch of the right common carotid.

(*b*) The superior profunda artery on the left arm was a branch of the posterior circumflex artery.

Abnormalities in other systems

(*a*) There was a small middle lobe of the left lung.

(*b*) The kidneys showed signs of persistent foetal lobulation.

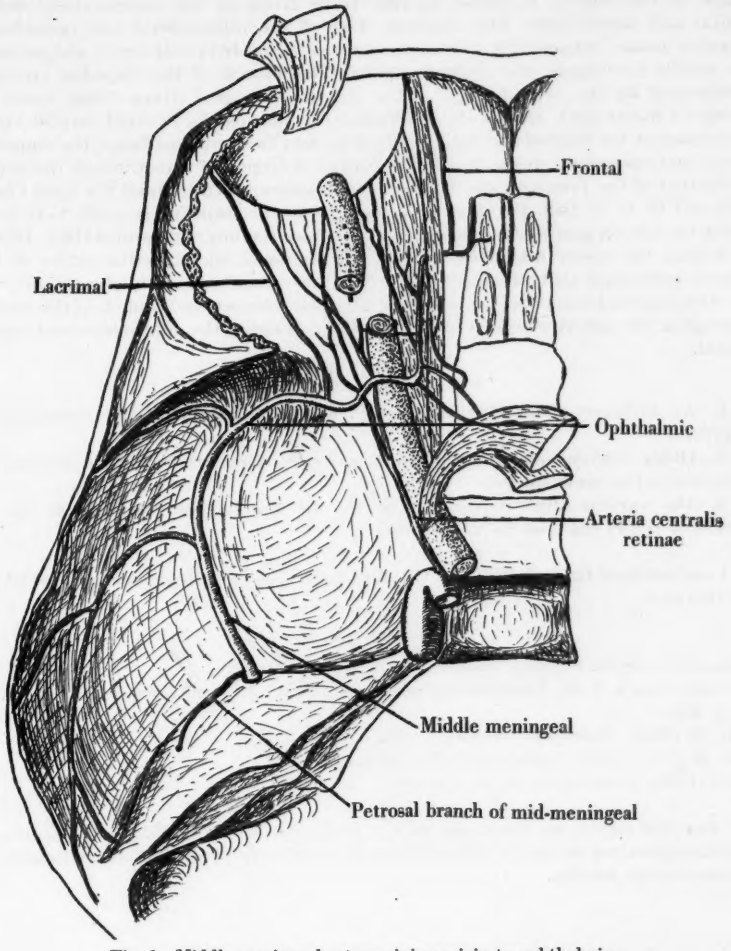

Fig. 1. Middle meningeal artery giving origin to ophthalmic.

Discussion

An abnormal ophthalmic artery from the middle meningeal is best explained as a persistence of the embryonic condition.

The primitive arrangement of the arteries in the region concerned here is susceptible of three interpretations: (i) the usually accepted view of the human embryologists, (ii) the state of affairs in the adult Madagascar lemurs, (iii) that seen in the lorisoids.

The current teaching concerning the arrangement of the vessels of this region in the human embryo is that given by Gray (1930), based on the researches of Tandler, viz. (i) a stapedial artery, arising from the anterior prolongation of the dorsal aorta near its junction with the 2nd branchial arterial arcade passes through the

hollow of the stapes. It breaks up into three divisions, viz. supra-orbital, infra-orbital and mandibular. The common stem of the infra-orbital and mandibular branches passes between the two roots of the auriculo-temporal nerve and becomes the middle meningeal; the original supra-orbital branch of the stapedial artery is represented by the orbital twigs of the middle meningeal (Gray, 1930, based on Tandler's researches). (ii) In the Madagascar lemuroids the internal carotid vessel terminates at the base of the skull by dividing into two terminal twigs, the stapedial artery and the arteria promontorii. The former is large and runs through the stapes to the roof of the tympanic cavity; the arteria promontorii is small (Le Gros Clark, 1934 and W. C. O. Hill, 1936). (iii) In the lorisoid the stapedial is small, as in man, whilst the arteria promontorii is large and becomes the internal carotid (Hill, 1936).[1]

Taking the above into consideration the course of the stapedial artery in the present case could be tentatively put forward as a combination of (i) and (iii), viz. the stylo-mastoid branch of the posterior auricular, the petrosal branch of the middle meningeal, the anterior branch of the middle meningeal, the ophthalmic and supra-orbital.

Conclusion

1. An instance of an abnormal artery arising from the middle meningeal is described.
2. Other vascular anomalies and some primitive characters in other systems are described in the same subject.
3. The various abnormalities discovered are tentatively explained as due to persistent embryonic and foetal conditions.

I am indebted to Prof. W. C. O. Hill for his invaluable help and advice in working out this case.

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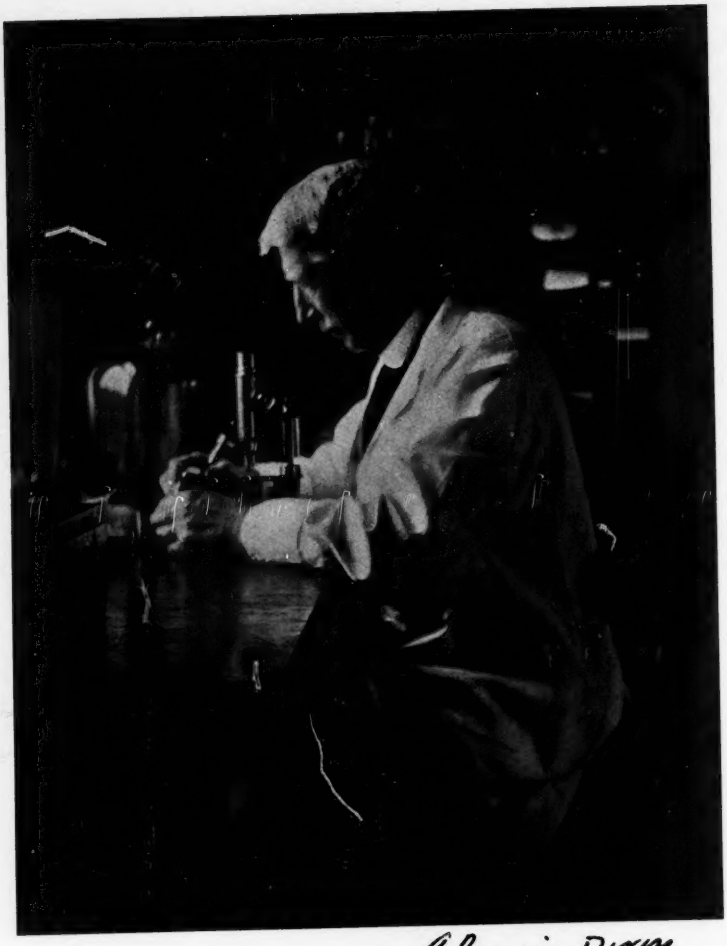
[1] Prof. Hill informs me that in the adult of *Loris* there is no stapedial artery. The internal carotid courses along the medial wall of the tympanic bulla and enters the cranium through the foramen lacerum medium.



A. Francis Dixon

IN MEMORIAM

PROFESSOR ANDREW FRANCIS DIXON, M.B., Sc.D. (DUBLIN)

By the death of Prof. Andrew Francis Dixon on 15 January 1936, at his home in Dublin, there is lost to the Anatomical Society one of its oldest, most beloved and most respected members.

Francis Dixon was born in Dublin in 1868, in, as he used to say with a twinkle in his eye, the National Maternity Hospital which now occupies the site of his old home. He was one of a family of eight, which included Prof. Stephen Dixon, lately retired from the Chair of Engineering in the Imperial College, South Kensington, and Prof. Henry Dixon, who still occupies the Chair of Botany in Trinity College, Dublin.

Dixon was educated, as a boy, in Rathmines, a Dublin suburb, at the school of Dr Benson, which has produced many great Irishmen, among them the late Prof. John Joly.

In 1885 he entered Trinity College, where his interests were chiefly with Natural Science, in which subject he gained a Senior Moderatorship and a Gold Medal; it was at this time that he came under the influence of Prof. D. J. Cunningham, who had been appointed to the Chair of Anatomy in Trinity College in 1883, Rev. Dr Samuel Haughton, and that great teacher, Dr John Mallet Purser.

In 1893 he received the Degree of M.B. of the University of Dublin, and it was in this year that he became a member of the Anatomical Society. It was in this year too, that he came to know another great and stimulating teacher when he went to Leipzig to attend the lectures of Prof. W. His. In the Obituary Notice of Prof. His which he wrote (1904) he gave expression to the great debt which so many anatomists owed to that indefatigable and enthusiastic worker.

On his return to Dublin, in 1894, Dixon was appointed to be Chief Demonstrator to Prof. Cunningham, with whom he thus became more closely associated and there began a friendship which lasted till Cunningham died in 1909.

In 1897 Dixon was appointed to the Chair of Anatomy at University College, Cardiff. He brought to Cardiff a stimulating enthusiasm and energy which was one of his most striking characteristics, and it was owing to him that an adequate supply of anatomical material was first made available in Cardiff. His work was done quietly and unobtrusively, and it is only now, when he is gone, that the debt to him, both in Cardiff and Dublin, is fully appreciated.

In 1903 Prof. Cunningham returned to Edinburgh, and his old pupil, Chief Demonstrator and friend, Francis Dixon, was appointed as his successor to the Chair of Anatomy in the University of Dublin. He left behind him, in

Cardiff, a record of work well done, of lasting friendships and of well-earned admiration and respect. To Trinity College, Dublin, he gave unstinted service for a period of 33 years. Dixon was an enthusiastic and stimulating teacher, and his very quiet, soft Irish voice and his great sense of humour made his lectures a delight. All his students loved him, and no old student ever came back to Trinity without looking up "Andy Frank", from whom they were always sure of a warm welcome and who at once knew their names and all that they were doing, or had ever done.

One of Francis Dixon's great interests was the Dublin Zoo. For many years he was its Honorary Secretary, and later its President, and, as in everything he did, he worked his hardest for it. He asked all his old students abroad, and any friend who was going abroad, to remember the Zoo and it is surprising to learn the number of animals that have reached the Zoo as a result of Dixon's enthusiasm. He was untiring too in his efforts to increase the membership of the Zoo and there are not a few people who have become members just for the love of Francis Dixon.

His work for the Anatomy School in Trinity College is more than can ever be told; he carried on the great work of his old Chief, maintaining the efficiency of the Department, helping and encouraging all who sought his advice, working unostentatiously for the benefit of the whole School, of which he was in later years, as Dean, the very pillar of foundation.

He was for many years the representative of the University of Dublin on the General Medical Council, and his administrative ability was no less than his academic; it was very largely due to that administrative ability that the Bicentenary Celebrations of the Medical School in Dublin were such an outstanding success in 1911.

Many other institutions in Ireland owe him a debt of gratitude, such as the Royal Irish Academy, The Royal College of Physicians, and the Alexandra College.

Many will recall the courteous way in which he conducted the Meetings of the Anatomical Society during his years of office as President, from 1933 to 1935, and it must be a great satisfaction to us all that he lived to receive so many of us in Dublin last summer, and none who were there will ever forget his kindly hospitality and careful thought for all our comforts and for the success of the Meeting, which he undoubtedly achieved.

The short list of some of Dixon's publications, attached to this notice, shows the wide range of his anatomical researches. It was in 1896 that he employed the glass plate method of reconstruction, which others have since found so useful, for his work on the development of the branches of the fifth cranial nerve. His work on the form of the empty bladder (1899–1900) is now a standard, and was incorporated, together with the results of his work on the supports of the uterus, in the section on the urogenital system, in Cunningham's *Text-book of Anatomy*, of which he was the original author, in 1902, while still in Cardiff. His discoveries of the markings on the surface of the calvarium in

different races (1904) are now quoted in the text-books of physical anthropology. There has seldom appeared a more perfect paper than Dixon's, on the architecture of the cancellous tissue forming the upper end of the femur (1910), and his account of the human oöcyte showing a first polar body is a model of industry and painstaking care, as one who saw him cutting the sections of what he called "the iron ovary" can testify.

But it is, above all, Dixon the man and the friend that so many will remember and miss. His big, loose-limbed body; the blue eyes that smiled so kindly and with such a warm welcome; the hearty grasp of his great hand that did such beautiful fine work, hands that did well and with all their might anything that he asked of them; the quiet, soft, shy voice and the great laugh at a good joke which no one enjoyed more or told better than he did.

His was a simple, upright and straightforward character, without any guile, and it was the simple things that he loved and of which he had such an amazing knowledge; the wind and the rain; the blue sky and the green fields; the sunshine and the sea and the clouds; the birds and the beasts and the flowers, and all the beauties of his native land; good hard manual labour and his pipe.

Francis Dixon was a man whom it was a privilege to know, and whose friendship was a priceless treasure.

May his memory never fade, but live as a shining example to every one of us who loved him dearly.

C. M. W.

SOME OF THE PUBLICATIONS OF PROF. A. FRANCIS DIXON

1896. "The development of the branches of the fifth cranial nerve." *Trans. roy. Dub. Soc.* ser. 2, vol. IV.

1897. "On the course of the taste fibres." *Edinb. med. J.* April.

1899. "The sensory distribution of the facial nerve in man." *J. Anat. Physiol.* vol. XXXIII.

1900. "The form of the empty bladder, and its connections with the peritoneum; together with a note on the form of the prostate." *J. Anat. Physiol.* vol. XXXIV.

1902. "The urogenital system." Cunningham's *Text-book of Anatomy.*

1904. "On certain markings due to nerves and blood vessels upon the cranial vault; their significance and the relative frequency of their occurrence in the different races of mankind." *J. Anat. Physiol.* vol. XXXVIII.
"Obituary Notice of Prof. W. His." *J. Anat. Physiol.* vol. XXXVIII.

1909. "In Memoriam. Daniel John Cunningham." *Dub. J. med. Sci.* August.

1910. "The architecture of the cancellous tissue forming the upper end of the femur." *J. Anat. Physiol.* vol. XLIV.

1911. "Supernumerary kidney. The occurrence of three kidneys in an adult male subject." *J. Anat. Physiol.* vol. XLV.

1912. *A Manual of Human Osteology.* Oxford Univ. Press.

1914. "Note on two cases of well marked suprasternal bones." *J. Anat. Physiol.* vol. XLVIII.

1918. "Why are the cerebral motor and sensory cortical areas arranged in an inverted order?" *Dub. J. med. Sci.* March.

1919. "The special supports of the uterus." *Dub. J. med. Sci.* April.

1927. "Human oöcyte showing first polar body." *Irish J. med. Sci.* ser. 6, No. 16.

REVIEWS

Brain Preparations, by means of defibrillation or blunt dissection. By J. Wilh. Hultkrantz. Translated from the first German edition by H. J. Wilkinson. (Medical Books, Ltd.) London: William Heinemann. 1935. Pp. vi+48. 4 text-figs. and 15 plates. Price 10*s.* 6*d.* net.

This book presents a full and ordered account of the method of dissection of the brain by defibrillation or blunt dissection, and some of the results to be obtained by this method are shown in the many illustrations, almost all of which are photographs of actual specimens.

Though the method is not new and has been used in England previously by Jamieson and by Green, as far as we know it has not been set out in book form as a laboratory manual until now.

The translation appears to be accurate and quite satisfactory, and the set up of the book is very good.

The Growth of the Surface Area of the Human Body. By Edith Boyd. (With a Foreword by Richard E. Scammon.) (University of Minnesota. The Institute of Child Welfare, Monograph Series, No. x.) (London: Oxford University Press.) 1935. Pp. x+145, figs. 50, tables 28. Price 22*s.* 6*d.* net.

This work, dedicated to John Abernethy, is a comprehensive and exhaustive study of a much-neglected branch of physical anthropology. It deals with the technique for the assessment of the surface area of the human body in both pre- and post-natal life. The author discusses in detail the various methods available for the evaluation of body area, both by direct measurement and by estimation, the mathematical formulae for relating this area to major bodily dimensions, and the inferences drawn from the growth phases of the area throughout life. Being written largely from the biophysical standpoint the work presupposes a familiarity with the tables, formulae and notations of the biometrician.

Mechanics of Normal and Pathological Locomotion in Man. By Arthur Steindler, M.D., F.A.C.S. (London: Baillière, Tindall and Cox.) 1935. Pp. xviii+424, 453 figs. Price 36*s.* net.

The first portion of this lengthy treatise deals with the general laws and mechanics of locomotion, the dynamics of bone and muscle action, and the physiology of co-ordination, fatigue and recovery. Its second, more extensive, portion treats of the special mechanics of normal locomotion and of functional derangements, the normal and abnormal articular apparatus of the spine, thorax, pelvis and major individual joints being discussed seriatim in considerable detail. Special attention is accorded to the mechanics and "pathomechanics" of posture and respiration. Final chapters discuss the normal gait under its various manifestations and analyse the abnormal gait of the crippled or infirm with a view to the possibility of conserving and increasing physiological function.

Both anatomist and orthopaedic surgeon will find this book a mine of information, though the abundance of statistical and mathematical tables may tend to obscure the author's story. The author has derived his information on bone physiology and anatomy mainly from German and American sources, and he has made but slight reference to the work which has deen done in this country.

The Cranial Muscles of Vertebrates. By F. H. Edgeworth, M.A., M.D., D.Sc. (Cambridge University Press.) 1935. Pp. viii + 493, Figs. 841. Price £5. 5*s.* net.

It is sometimes given to a man, if he be among the favoured, himself to gather his life's work into an ordered whole and make a gift of it to his subject. It is for such a gift that the Science of Morphology is indebted to Prof. Edgeworth. His book is the record of his work "...of many years, as far as medical practice and teaching permitted"; now it is systematically arranged, its evidence is compared with the work of the last 150 years, and its conclusions are discovered and discussed. It is impossible not to be impressed by the work or to appreciate the achievement of the book, for it is the most comprehensive and masterly study of the cranial muscles that has appeared. But it is not only that; it is a vindication of the conception of structure that is realized by the study of structure and its development and comparison.

Two introductory chapters of the book treat of the relationship of the muscle plates of the head to the anterior myotomes and the enumeration of the branchial segments. They necessarily deal with a highly specialized and controversial subject, the origin and development of the mesoderm of the head; and, as is to be expected, they treat it not more fully than to establish a generalized conception of the relationship and a possible interpretation of the varying branchial number. There emerge from them, however, the developmental bases of the classification of the cranial muscles in six groups. The following eighteen chapters, the main part of the book, consist of descriptions of the single muscles of the groups, group by group, through the orders of the vertebrates, order by order; their arrangement, development, and motor innervation are described, and their homology, evident or probable, is discussed. There can be nothing but admiration and praise for this part of the book: As judged by the muscles with which the reviewer is acquainted it is a complete account of the knowledge there is of the muscles of the head and their motor nerves; it contains references to some 900 papers, and it is pointed with 841 diagrams that have meaning; and, as must be when a work on morphology is complete, it realizes where knowledge ends and where the significant absences of it lie, and it is full of suggestions of work that waits to be done. How little is known, for example, of the processes of the spinal extension of the nucleus of the vagus, how ununderstandable is the nerve supply of the hyoepiglotticus of the elephant, and how difficult it often is to determine homology and even what the determining criteria are to be, Prof. Edgeworth makes us aware; he gives 39 pages of synonyms of the cranial muscles and he tells us "it is far from complete". These are great services to have given to his subject; their reward will be the use of his book as the authoritative reference of its time and the stimulus of many a worker.

The attempt is then made, in the last chapters of the book, to define "...as far as possible" the nature of the primitive musculature in the vertebrate orders and on this evidence to determine their genetic relationship. This is in the tradition of morphology and, though the conclusions to which Prof. Edgeworth comes will require to be examined and may even need to be revised, it is the method of morphology. His evidence, as he says in his last words, "has not hitherto been utilized in attempting to solve the difficult problems of phylogeny". The value of the evidence of muscles, which are but part of a part, must always be critically considered, for, as Hunter said, "the form and size of muscles are adapted to the uses in which they are employed and these in general arise from the nature of the parts or joints to be moved". That is the summation of morphology; it recognizes, as Prof. Edgeworth has recognized, the plasticity of structure and its variability, for it is founded, as his work is, on the whole breadth of comparison; and in the end it meets its difficulties and faces them for, in his words, it is "in some unknown way the growing organism appears to foresee its future needs" and there is a development of new things from old. It is

pure teleology; but it is of the highest importance that a book which treats of structure in the way of structure and follows the way to the end should have appeared in English at the present day, and, we hope, it is not without significance.

The following books have been received:

Synopsis of Surgical Anatomy. 3rd Edit. By ALEXANDER LEE MCGREGOR. With a foreword by Sir Harold Stiles, K.B.E. (Bristol: John Wright & Sons Ltd.) 1936. Pp. xvii+664, 648 figs. Price 17*s.* 6*d.* net.

Baillière's Synthetic Anatomy. By J. E. Cheesman. Complete in 14 parts. (London: Baillière, Tindall & Cox.) 1936. Price 3*s.* each part.

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